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PAKOWKI LAKE WATER QUALITY STUDY (1996)

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EXECUTIVE SUMMARY

Pakowki Lake is an intermittent, hypertrophic lake in southeastern Alberta. Over 200,000 waterfowl are believed to have died at Pakowki Lake during a severe outbreak of avian botulism in 1995. A water quality sampling program was implemented in 1996 to determine whether the physical, chemical and biological characteristics of this lake influence the timing and severity of these outbreaks. In particular, this study was designed to determine whether there was evidence that cyanobacterial blooms increase the rate of avian mortality.

Avian mortality due to botulism began about July 17-19 and peak mortality occurred in mid-September in both 1995 and 1996. During both years, avian mortality rates were lower in an impounded bay than in the central basin of Pakowki Lake.

Clostridium botulinum is an obligate anaerobe, and the rate of toxin production by this organism is strongly influenced by water temperature in the lake. Instruments that recorded continuous temperature, oxygen, conductivity and pH were installed from June 12-September 19, 1996. Reliable oxygen data were not collected in the central basin during the period of onset of avian botulism (around July 19), but data from the impounded bay were deemed acceptable. Nocturnal oxygen depletion began in the impounded bay about August 2, 1996. Water temperature in both of the sampled basins exceeded 20°C prior to the onset of avian botulism. This is the temperature considered necessary for the rapid formation of *Clostridium botulinum* type C toxin. However, water temperature was much lower, generally in the range of 10° to 17°C, when peak avian mortality occurred in mid-September. Research has found that toxin production declines at lower temperatures. However, the toxin is relatively stable and could therefore persist and accumulate over the summer, causing still higher mortality in mid-September.

Pakowki Lake and two major tributaries were sampled for a wide range of water quality variables from May 28-October 10, 1996. Phosphorus and chlorophyll *a* levels place Pakowki Lake at the upper end of the hypertrophic range of algal productivity. Levels of suspended sediment and non-algal turbidity were extremely high, compared to other Alberta lakes, but declined prior to a severe algal bloom in August. The cyanobacterial bloom in Pakowki Lake collapsed after August 20. The collapse of the algal bloom preceded a dramatic increase in avian mortality rates in mid-September. In the central basin, the algal bloom was dominated by the cyanobacterium *Aphanizomenon flos-aquae*, which can produce various potent neurotoxins. Algal samples were tested for one of these neurotoxins, anatoxin-a, but none was detected. Bioassays on mice were used to determine that a neurotoxin, other than *Clostridium botulinum* type C neurotoxin, was present in algal samples collected on September 19 during the period of peak avian mortality. However, this neurotoxin has not been identified, and the extent to which it caused or influenced avian mortality in 1996 is not known.

A mass balance analysis for Pakowki Lake indicated that a large amount of total phosphorus was released from internal sources (e.g., lake sediments) prior to the severe algal bloom in August 1996. The analysis also determined that about 89.5% of all total phosphorus loading to

Pakowki Lake during February 20-October 10, 1996 was from internal sources. Only 8.6% of the total phosphorus loading to Pakowki Lake in 1996 was loading from six tributaries, and of that Etzikom Coulee contributed about 59%.

Dissolved solids, pH, sodium adsorption ratio, sodium, sulphate, and several metals in Pakowki Lake samples exceeded water quality guidelines for agricultural use or the protection of aquatic life. High levels of these substances may influence lake management options. For example, Pakowki Lake water would not be suitable for the irrigation of some crops. However, there is no evidence that these constituents increased the incidence of avian mortality.

A preliminary model for the development of avian botulism in Pakowki Lake is proposed. This model relates the physical, chemical or biological characteristics of this lake to the onset and maintenance of an avian botulism outbreak. The 1996 results apparently provide the first empirical evidence of a link between algal production, cyanobacterial toxins and avian mortality due to botulism, which must be confirmed by additional sampling. If cyanobacterial toxins cause a significant portion of the avian mortality in this lake, then reducing the level of algal productivity might help control avian mortality. Various investigations are required before lake management options to reduce algal productivity can be evaluated.

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

Pakowki Lake is a large (about 109 km^2 in 1996) terminal water body in southeastern Alberta (49°20' 45" N; 110°55' 40" W). The lake is intermittent and has a mean depth of 1.2 m. It was essentially dry for part of the 1980's, but began to refill in the early 1990's.

Pakowki Lake has a history of avian botulism, with reports of outbreaks as early as 1928. *Clostridium botulinum* type C, the most frequent cause of avian botulism, was confirmed in 1995 using a mouse inoculation test (Pybus and Eslinger 1996). Botulism outbreaks occurred in 1975, 1980, 1994 and the most severe outbreak occurred in 1995, when 100,825 carcasses were collected during efforts to control the outbreak (Pybus and Eslinger 1996). Total losses that year were thought to exceed 200,000 waterfowl. Ducks Unlimited Canada has estimated that the total loss of ducks at Pakowki Lake in 1995 was more than was produced in North American Waterfowl Management Plan projects in prairie Alberta that year (Neraasen 1997).

The physical, chemical and biological characteristics of a lake may influence the timing and severity of botulism outbreaks, but the role of these environmental factors is poorly understood and there have been no previous studies in Alberta. *Clostridium botulinum* is an obligate anaerobe (Rosen 1971), and the initial outbreak of avian botulism in lakes appears to be associated with low redox in lake sediments. However, Bell *et al.* (1955) found that this anaerobe can also survive and produce toxin in insect larvae kept in oxygenated water. Haagsma (1974) found that toxin was not produced by *Clostridium botulinum* type C at temperatures less than 10°C. Although detectable levels of toxin were produced at 15°C, there was a faster rate of production at higher temperatures and potentially lethal concentrations were produced in nine days at 20°C. Rocke (1997) recently surveyed 32 wetlands with avian botulism in the United States, each paired with a site without the disease. High risk sites were observed to be moderately saline (1,000-4,000 mg/L TDS), low in sediment redox and had neutral to alkaline pH.

It has been noted that avian botulism outbreaks often occur in lakes that experience cyanobacterial blooms; a bloom was noted during the first recorded outbreak of botulism at Lake Newell (Alberta) in 1925-26 (Munro 1927, cited in Pybus and Eslinger 1996). However, cyanobacterial toxins have never been measured during botulism outbreaks, and a relationship between cyanobacteria and botulism has not been established. Hypothetically, cyanobacterial toxins may kill birds, or may weaken them and thus increase susceptibility to botulinum toxin (Pybus and Eslinger 1996). Some cyanobacterial neurotoxins could cause identical symptoms to botulinum poisoning (M. Pybus, 1996; personal communication). Another possible mechanism of cyanobacterial influence has been proposed by Murphy *et al.* (1996). Calcium carbonate formed in alkaline water late in a cyanobacterial bloom may precipitate algal cells, which coat the lake sediments and induce low redox conditions that favour the growth of *Clostridium botulinum*.

1.1 STUDY OBJECTIVES

The Pakowki Lake Working Group was formed in Alberta in 1995, with representatives of various agencies and disciplines. The goal of this Working Group is to investigate and implement methods of limiting avian mortality at Pakowki Lake. Due to the suspected link between water quality and avian botulism, the Water Sciences Branch of Alberta Environmental Protection (AEP) was asked by the Working Group to conduct a water quality sampling program during 1996. Few historical data were available. The program was designed to meet the following specific objectives:

(a) to measure physical and chemical variables that may be correlated with the development of avian botulism;

(b) to investigate the role of cyanobacteria, and cyanobacterial toxins, in the onset and severity of avian botulism at Pakowki Lake;

(c) to document the trophic state of Pakowki Lake, sample major tributaries and develop a preliminary phosphorus budget for the lake, to guide planning for lake management;

(d) to measure pesticides, herbicides and other potential toxins in major tributaries and in Pakowki Lake, to determine if these compounds could reach levels acutely toxic to birds.

Pybus and Eslinger (1996) reported that the 1995 botulism outbreak began in the northwest arm of Pakowki Lake, east of a Ducks Unlimited Canada earthen berm (Figure 1), but there was little mortality in the impounded area west of this berm. Of the 100,825 carcasses collected over the entire lake, “probably less than 6" were found west of the berm. Pybus and Eslinger (1996) reported that in 1995 the weir in this berm was holding back no more than 1 m of head in the 0.7 km^2 impounded area. The 1996 sampling program at Pakowki Lake included routine

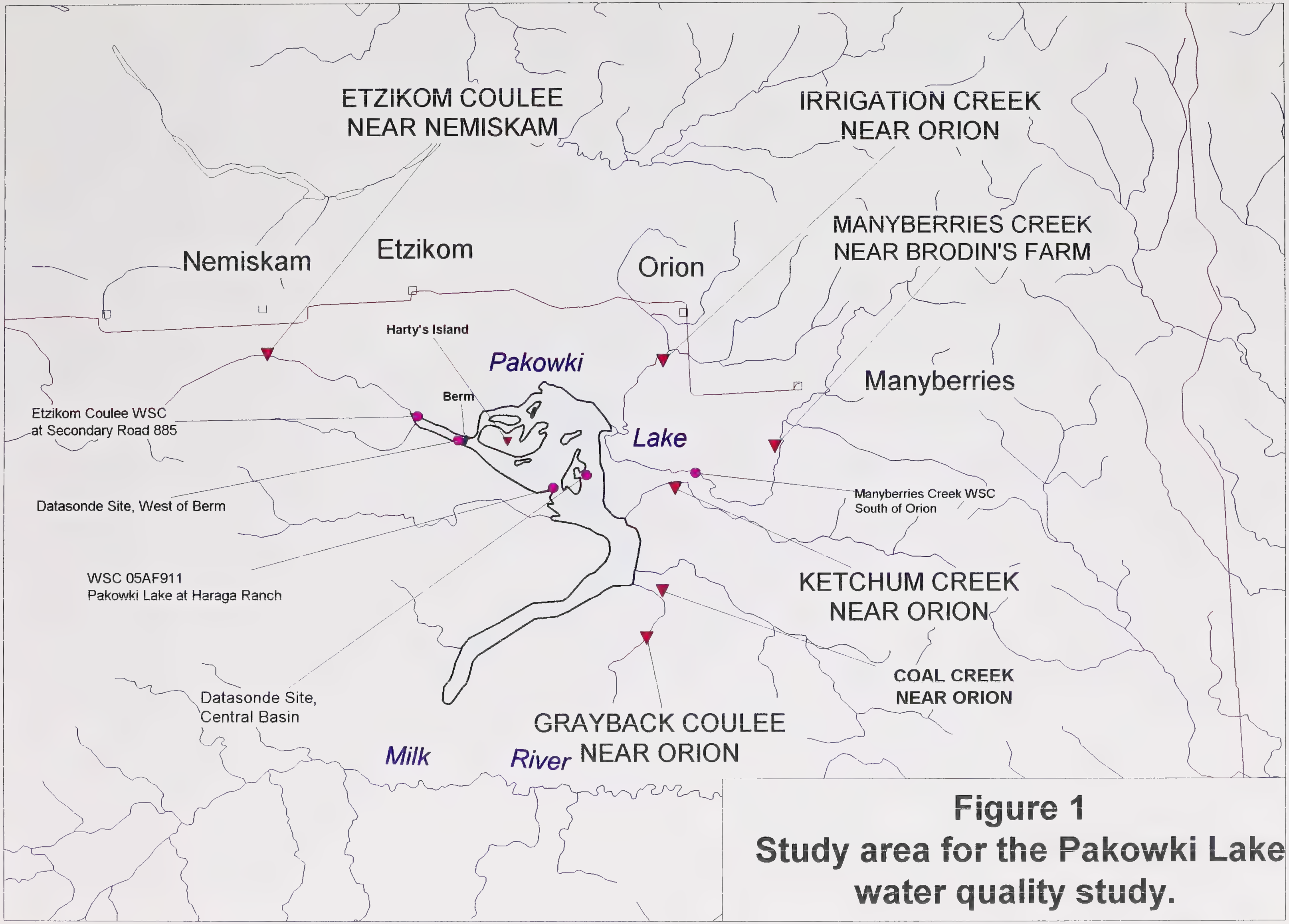

ETZIKOM COULEE NEAR NEMISKAM
IRRIGATION CREEK NEAR ORION
MANYBERRIES CREEK NEAR BRODIN'S FARM
Nemiskam
Etzikom
Orion
Manyberries
Harty's Island
Pakowki
Berm
Lake
Etzikom Coulee WSC at Secondary Road 885
Datasonde Site, West of Berm
Manyberries Creek WSC South of Orion
WSC 05AF911 Pakowki Lake at Haraga Ranch
KETCHUM CREEK NEAR ORION
COAL CREEK NEAR ORION
Datasonde Site, Central Basin
GRAYBACK COULEE NEAR ORION
Milk
River

Figure 1
Study area for the Pakowki Lake water quality study.

sampling of physical, chemical and biological variables in the central basin and in the bay west of the berm, as a low mortality control area. The results of the 1996 water quality sampling program at Pakowki Lake are presented in this report.

2.0 MATERIALS AND METHODS

All lake and tributary sampling followed field methods described in Alberta Environmental Protection (1993*a*). Pakowki Lake was sampled at fortnightly intervals from June 12 to August 20, and otherwise at a monthly interval from May 28 to October 10, 1996. The lake was sampled as two basins, divided by the berm on the northwest arm (Figure 1). Composites of subsurface grab samples from 10 random sites in each of the two lake basins were prepared, and grab samples were collected at a site on Etzikom Coulee and Manyberries Creek (Figure 1) on each sampling date. Because all sampling sites in the lake were extremely shallow, subsurface grab samples were collected rather than the vertically-integrated euphotic zone samples normally collected from lakes. Light penetration near the datasonde sites was measured with an underwater photometer (Li-Cor Li 1000 Lightmeter with 4424 Sensor). Composite samples from the central basin in Pakowki Lake, and grab samples from Etzikom Coulee and Manyberries Creek, were collected October 10 for herbicide and pesticide scans.

Duplicate subsamples from the lake composites were collected each month for phytoplankton analysis and preserved with Lugol's solution. Algal species were identified, cell numbers counted and biomass estimates were prepared by Dr. M. Agbeti of Bio-Limno Research and Consulting (Appendix II). Samples were counted and identified using a Utermohl's sedimentation chamber. Samples were settled overnight and a minimum of 400 individual algal cells, colonies or filaments were counted in transects. Biomass estimates were then prepared from the counts using published cell biomass values. A single duplicate sample was identified by Dr. C. Earle, Concordia University College, for quality assurance (Appendix II).

Algal samples were collected for toxin analysis using horizontal hauls of a 63 micron mesh 0.18 m x 0.48 m zooplankton net near the datasonde site in each lake basin. These algal samples were frozen as a composite and later freeze-dried by B. Kotak at the University of Alberta. No attempt was made to separate zooplankton from the algal material in each sample.

A calibrated Datasonde II meter was installed at one site just west of the berm in the northwest bay, and another meter was installed near the deepest area (1.5 m depth) in the central basin (Figure 1). These units recorded temperature, dissolved oxygen, pH and conductivity near the middle of the water column every half-hour from June 12 to September 19, 1996. Measurements by the datasondes were regularly verified by field measurements (generally 11:00-14:30) of pH, temperature and conductivity, using another calibrated Hydrolab meter, and dissolved oxygen was verified by Winkler titration.

Dissolved oxygen and pH data exceeded acceptance criteria for the datasonde in the central basin from 22:00, June 15 to 09:00, August 7. Therefore, these data were deleted from the record. The electrodes for these variables appear to have been adversely affected by the high levels of suspended sediment at this site. Redox in lake water was also measured at the site in the central basin from August 20 to September 19 only, but these data did not meet acceptance criteria.

Daily precipitation data for 1996 from the Environment Canada weather station at Aden, about 25 km southwest of Pakowki Lake were supplied by Alberta Agriculture, Food and Rural Development. Wind velocity data were obtained from the Agriculture and Agri-Food Canada weather station at Onefour, about 30 km southeast of Pakowki Lake.

2.1 CHEMICAL ANALYSES

Samples were analyzed for inorganic variables at Chemex Labs Alberta Inc., and other samples were analyzed for herbicides and pesticides at the Alberta Environmental Centre. Additional phosphorus analysis (method 15422 and 15114, Table 1) and chlorophyll *a* analyses were conducted by the Monitoring Branch, AEP at the MacIntyre Centre in Edmonton. All method codes, full variable names, method detection limits and method descriptions are listed in Table 1. Subsamples of freeze-dried algal material were analyzed for anatoxin-a by S. Kenefick at the Okanagan University College using gas chromatography-mass spectrometry, as described in Appendix III.

Table 1. Summary of analytical methods used in the 1996 water quality program at Pakowki Lake.

VMV CODE	VARIABLE NAME	MDL	UNIT	METHOD DESCRIPTION
Nutrients				
7505	ammonia total	0.01	mg/L	colorimetrically (berthelot method)
7110	nitrogen dissolved (nitrite/nitrate)	0.003	mg/L	colorimetry on autoanalyzer
7602	nitrogen total (calcd.)	0.02	mg/L	calculated method
7015	nitrogen total Kjeldahl	0.05	mg/L	colorimetrically (berthelot method)
15422	phosphorous total (AEP lab)	0.001	mg/L	colourimetry with ammoniium molyd.; hydrazine; etc.
15114	phosphorous total dissolved (AEP lab)	0.002	mg/L	colourimetry with ammoniium molyd.; hydrazine; etc.
15406	phosphorous total (Chemex)	0.003	mg/L	colourimetry with ammonium molybdate; etc.
15423	phosphorous total dissolved (Chemex)	0.003	mg/L	the unfiltered sample is digested in an autoclave using a sulfuric
14106	silica reactive	0.05	mg/L	colourimetry using heteropoly blue method(autoanalyzer)
Miscellaneous Variables				
10151	alkalinity phenolphthalein CaCO$_3$	0.1	mg/L	potentiometric titration
10111	alkalinity total CaCO$_3$	0.5	mg/L	titro processor
6201	bicarbonate (calcd.)	0.5	mg/L	calculated value
6104	carbon dissolved organic	0.2	mg/L	infrared analysis
6301	carbonate (calcd.)	0.5	mg/L	calculated value
6715	chlorophyll a	0.1	µg/L	fluorometry
2021	colour true	5	rel units	visual comparison
10602	hardness total (calcd.) CaCO$_3$	0.5	mg/L	calculated method
8501	hydroxide (calcd.)	0.5	mg/L	calculated method
10301	pH lab	0.01	pH units	electrometric method
6537	phenolic material	0.001	mg/L	automated 4-aminoantipyrine colorimetric method
10451	residue filtrable (TDS measured)	1	mg/L	gravimetric method
10501	residue fixed nonfiltrable	0.4	mg/L	gravimetric method
10401	residue nonfiltrable	0.4	mg/L	gravimetric method
201	total dissolved solids (TDS calcd.)	1	mg/L	calculated value
2074	turbidity	0.1	NTU	nephelometric method using a hach turbidimeter; units ntu
Major Ions				
20111	calcium dissolved/filtered	0.01	mg/L	inductively coupled argon plasma emission spectroscopy
17206	chloride dissolved	0.5	mg/L	colourimetry on autoanalyzer with ferric nitrate and mercury thiocyanate
12111	magnesium dissolved/filtered	0.01	mg/L	inductively coupled argon plasma emission spectroscopy
19111	potassium dissolved/filtered	0.02	mg/L	inductively coupled argon plasma emission spectroscopy
11111	sodium dissolved/filtered	0.01	mg/L	inductively coupled argon plasma emission spectroscopy
16309	sulphate dissolved	0.1	mg/L	ion chromatography
16101	sulphide dissolved	0.005	mg/L	methylene blue colorimetry, automated gas dialysis
Metals				
13311	aluminum extractable	0.01	mg/L	inductively coupled argon plasma emission spectrometry
33005	arsenic extractable	0.0002	mg/L	flameless atomic absorption
5211	boron extractable	0.01	mg/L	inductively coupled argon plasma emission spectrometry
48316	cadmium extractable	0.0002	mg/L	inductively coupled mass spectrometry
24360	chromium extractable	0.002	mg/L	inductively coupled argon. emission spectrometry
29316	copper extractable	0.0002	mg/L	inductively coupled mass spectrometry
26311	iron extractable	0.01	mg/L	inductively coupled argon plasma emission spectrometry
82316	lead extractable	0.0003	mg/L	inductively coupled mass spectrometry
25311	manganese extractable	0.001	mg/L	inductively coupled argon plasma emission spectrometry
80011	mercury total	0.05	µg/L	flameless atomic absorption on an autoanalyzer
28317	nickel extractable	0.0005	mg/L	inductively coupled mass spectrometry
34005	selenium extractable	0.0002	mg/L	flameless atomic absorption
23311	vanadium extractable	0.002	mg/L	inductively coupled argon plasma emission spectrometry
30501	zinc extractable	0.001	mg/L	inductively coupled argon emission spectrometry
Field Variables				
100922	oxygen dissolved	0.01	mg/L	oxygen, dissolved: measured by electronic meter with a polarograph
8101	oxygen dissolved	0.01	mg/L	winkler method (azide modification)
100923	pH	0.01	pH unit	pH: measured by electronic meter with a glass pH reference electrode
100924	specific conductance	0.2	µS/cm	specific conductance: measured by electronic meter with nickel cell
100925	temperature water	0.1	deg C	temperature, water: measured by electronic meter with a thermistor
2079	light intensity	1	ft candle	downwelling illuminance measured with underwater photometer

2.2 GUIDELINE EVALUATION

All data were compared to the most stringent available water quality guidelines for water uses that occur in the Pakowki Lake basin (Section 3.3, Table 2). The lowest of either the Alberta Ambient Surface Water Quality Interim Guidelines (ASWQG) (AEP*b* 1993) or the Canadian Water Quality Guidelines (CWQG) (CCME 1995) for freshwater aquatic life and agriculture were used in this evaluation. In the case of copper, a draft guideline for Alberta (Shaw 1996) was used.

2.3 DATA ANALYSIS

Mass balance analysis was used to provide a preliminary phosphorus budget for the lake, which accounted for all significant phosphorus loading from internal sources (including: sediments and groundwater) and external sources (tributaries, diffuse runoff and atmospheric deposition). Since Pakowki Lake is a terminal basin, phosphorus loss to an outflow stream was not required in the mass balance calculation. Net internal phosphorus loading (or deposition) in Pakowki Lake was estimated using a conventional mass balance equation, where for each sampling interval:

Deposition/Release = outflow P load - (atmospheric P-loading to lake surface^{a} + sum of all stream loadings^{b} + diffuse area loadings^{c}) +/- (change in lake P mass^{d})

a = P mass deposited directly on the surface of Pakowki Lake, with area adjusted for changes in lake level according to the area-capacity relationship in Appendix A of Wohl (1996). Precipitation volumes were estimated from precipitation data from the Environment Canada weather station at Aden. Precipitation phosphorus concentration data from the Eagle Lake watershed in 1988 was used with volumes to estimate phosphorus mass;

b = estimated using May 28-October 10 TP concentration for Etzikom Coulee and Manyberries Creek and *preliminary* daily mean flows from Water Survey of Canada sites (Station No. 05AF905 and 05AF010, respectively). Manyberries Creek loadings were then apportioned to ungauged tributaries (Irrigation Ck., Ketchum Ck., Canal Ck. and Coal Ck.) for each interval based on flow volumes, which were estimated from the total season volume for Manyberries Creek using watershed area ratios from Appendix A (p. 31) of Wohl (1996);

c = areas of diffuse runoff, without discrete channels, were included in watershed areas for ungauged tributaries in Appendix A of Wohl (1996)(A. DeBoer, personal communication);

d = water level on each sampling date was interpolated (Section 3.4.1, Table 3) between water level measurements at Station No. 05AF911 (location in Figure 1), and used to calculate lake volume using the area-capacity relationship in Appendix A of Wohl (1996). Lake TP mass was then estimated from lake volume and composite TP concentration for the central basin each sampling day.

A positive residual was taken as an estimate of net internal loading; a negative value was taken as an estimate of deposition. Groundwater loading or losses are not separately included in this calculation, as accurate estimates are not available, but would be included in the residual. Sampling interval in the mass balance calculation refers to loading during days prior to and including each lake sampling day. Approximate tributary loadings prior to May 28, 1996, when sampling began, were estimated using median total phosphorus concentrations for Etzikom Coulee and Manyberries Creek and measured flows for the period February 20-May 27, and Manyberries Creek loadings were apportioned to the ungauged tributaries. Similarly, precipitation total phosphorus loads for the time period before the sampling program (February 20-May 27) were estimated using precipitation data from the Aden weather station and early spring phosphorus concentration measured in precipitation at Eagle Lake in 1988 (unpublished data, Water Sciences Br., AEP).

3.0 RESULTS AND DISCUSSION

3.1 AVIAN MORTALITY

The number of dead birds collected each day by field crews during 1996, west of the berm and in the central basin of Pakowki Lake, was supplied by Mike Anderson of Ducks Unlimited Canada. Between July 19 and October 15, a total of 12,225 dead birds were collected in the central basin (108.3 km^2 at 856.0 m elevation above sea level), and 58 were found west of the berm (area of 0.7 km^2) between July 22 and October 7. The total number of dead birds collected in 1996 was therefore much less than the 100,825 birds collected in 1995. However, it should be noted that the dates when mortality was first noted (July 17 in 1995, July 19 in 1996) and period of peak mortality (first three weeks of September) in the two years were virtually identical (Pybus and Eslinger 1996; Figure 2). Furthermore, in both years, the initial outbreak began in the northwest arm, adjacent to a group of islands in that arm that are locally known as Harty's Islands (Figure 1). Similarities in timing and spatial distribution of mortality in the two years strongly suggests that environmental factors influence the onset and development of a botulism outbreak in Pakowki Lake.

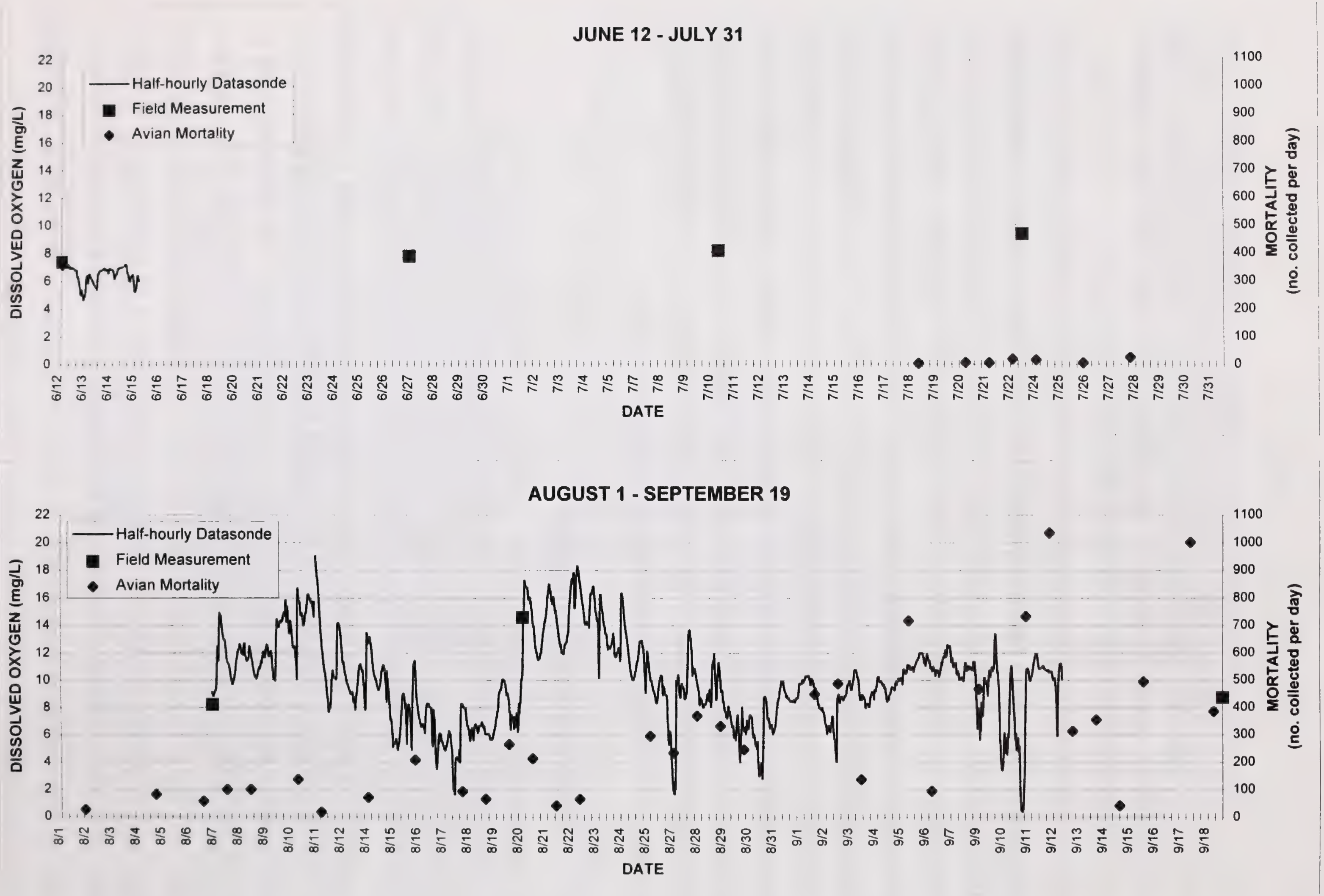


Figure 2. Dissolved oxygen and avian mortality in Pakowki Lake central basin, June 12-September 19, 1996.

The water quality sampling program was designed to test the hypothesis that mortality from avian botulism would be lower under the physical, chemical and biological conditions found in the impounded area west of the berm, than in the central basin. Expressed as carcasses per unit area, there were fewer birds collected west of the berm in 1996 (83/km^2)(Figure 3), than in the central basin (113/km^2). Various factors complicate comparisons between mortality rates in the two basins in 1996. Firstly, lake levels were apparently higher in both sampling areas in 1996, than in 1995 (M. Anderson, personal communication), which may have influenced avian mortality in both basins. Furthermore, since the central basin is much larger than the small bay west of the berm, it would be very difficult to search both areas for carcasses with the same level of effort. Finally, it is not known if any of the birds found west of the berm moved to that area after first contacting botulism in the central basin.

3.2 OXYGEN, TEMPERATURE and pH

Oxygen, temperature and pH at the two datasonde installations are plotted with avian mortality in Figures 2 to 5 to determine if these variables were correlated with mortality. Only data verified by independent field measurements have been included in these figures. The available data suggest that both sites were well-oxygenated early in the summer, with brief periods of nocturnal oxygen depletion and extreme supersaturation in August (Figures 2 and 3). Anoxic conditions could well have occurred in bays, or at the sediment/water interface, during periods when the mid-water column sites were well-oxygenated. A small number (n=3) of sulphide samples were collected from both areas late in the summer (Appendix I). These were all less than the detection limit, which also suggests that the lake was not anoxic for extended periods.

Unfortunately, the continuous oxygen data from the central basin were not considered reliable during the interval from late 22:00, June 15 to 09:00, August 7, which includes the date (July 19) when avian mortality was first observed. However, pronounced fluctuation in dissolved oxygen began on July 20 (minimum of 2.7 mg/L) in the basin west of the berm (Figure 3), about the same time that the first avian mortality was reported in the central basin. Nocturnal oxygen depletion in lake water west of the berm occurred August 2-7 (Figure 3), and sediments were probably anaerobic even earlier (Marsden 1989). Dissolved oxygen was relatively high in the central

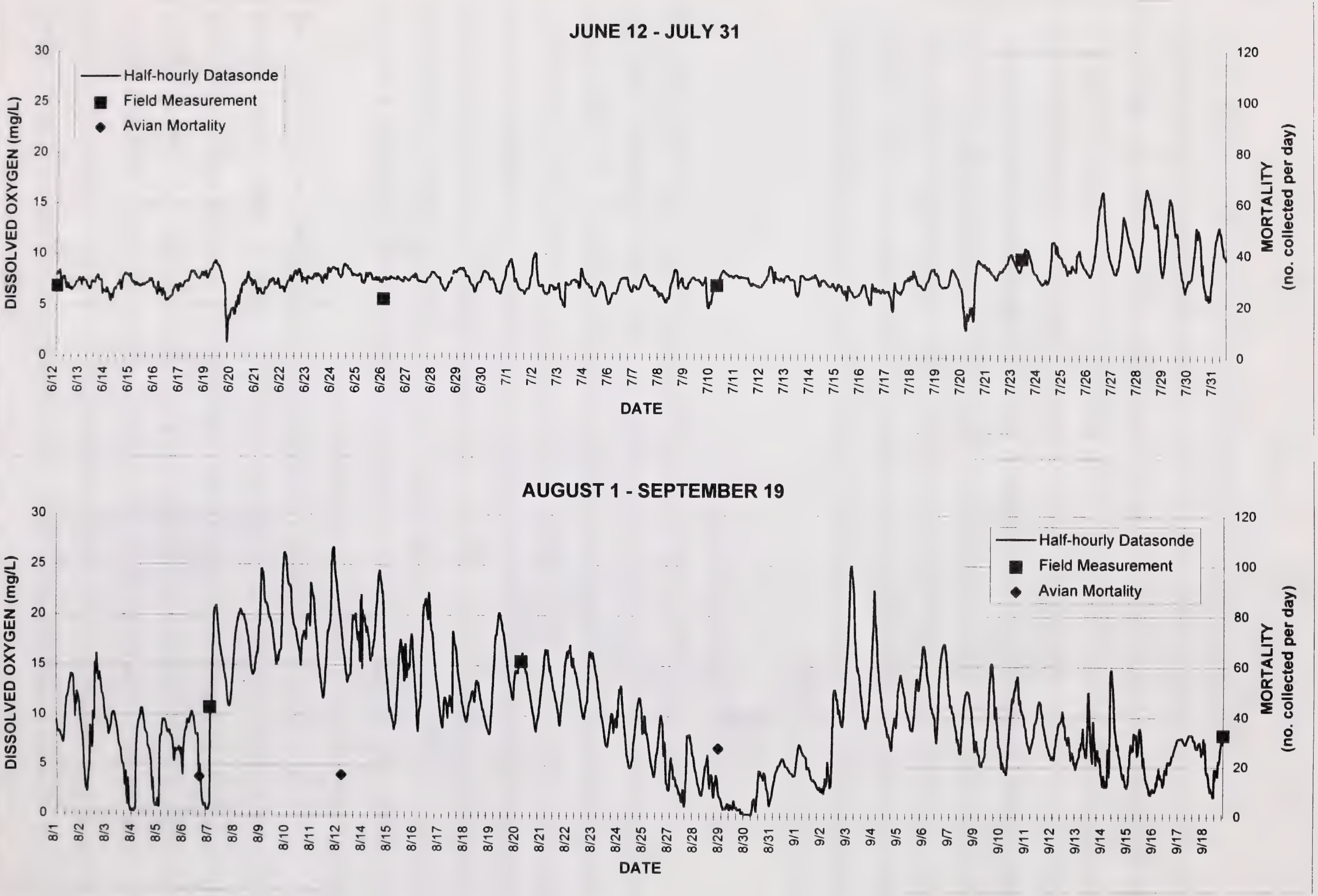


Figure 3. Dissolved oxygen and avian mortality in Pakowki Lake northwest bay, June 12-September 19, 1996.

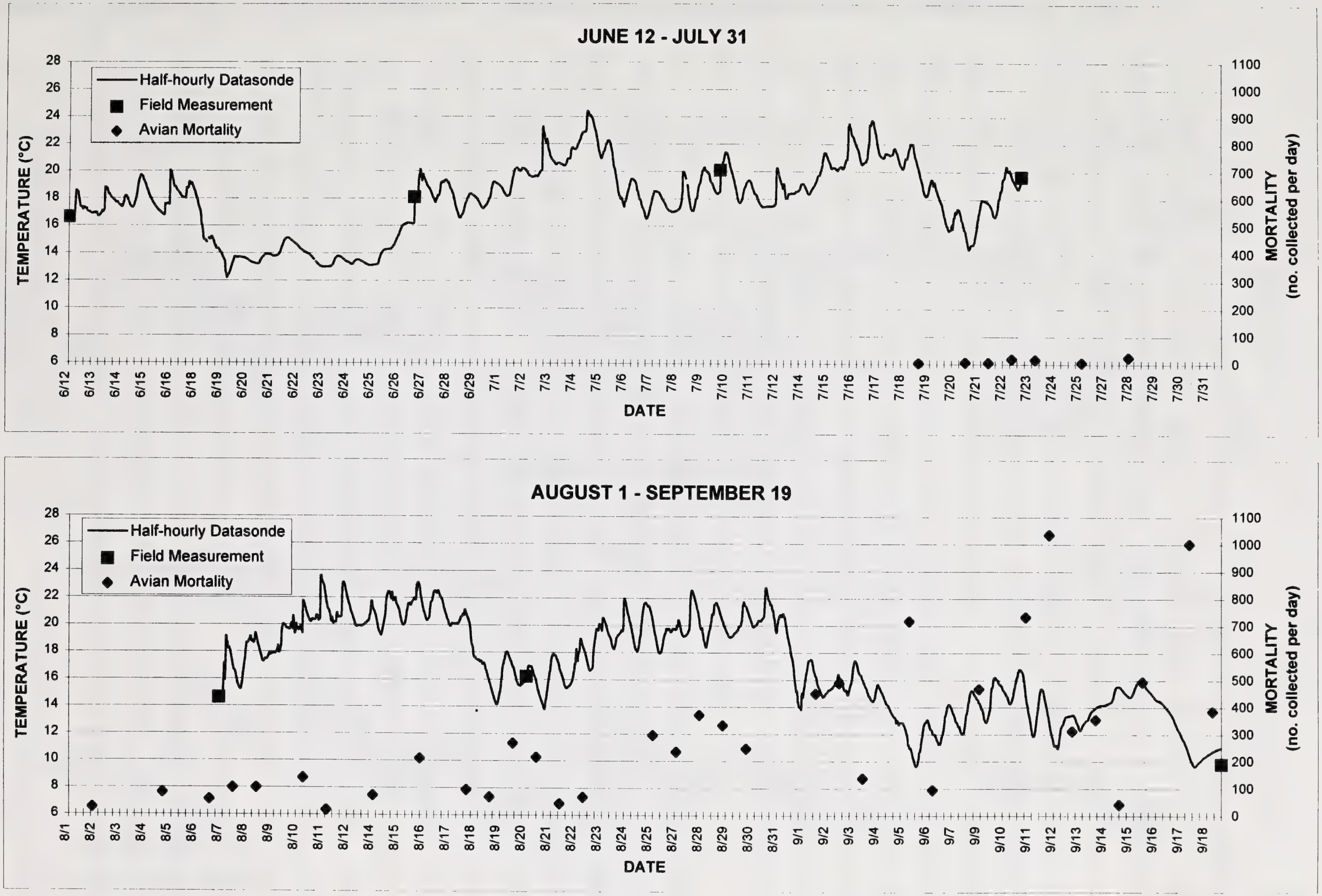


Figure 4. Temperature and avian mortality in Pakowki Lake central basin, June 12-September 19, 1996.

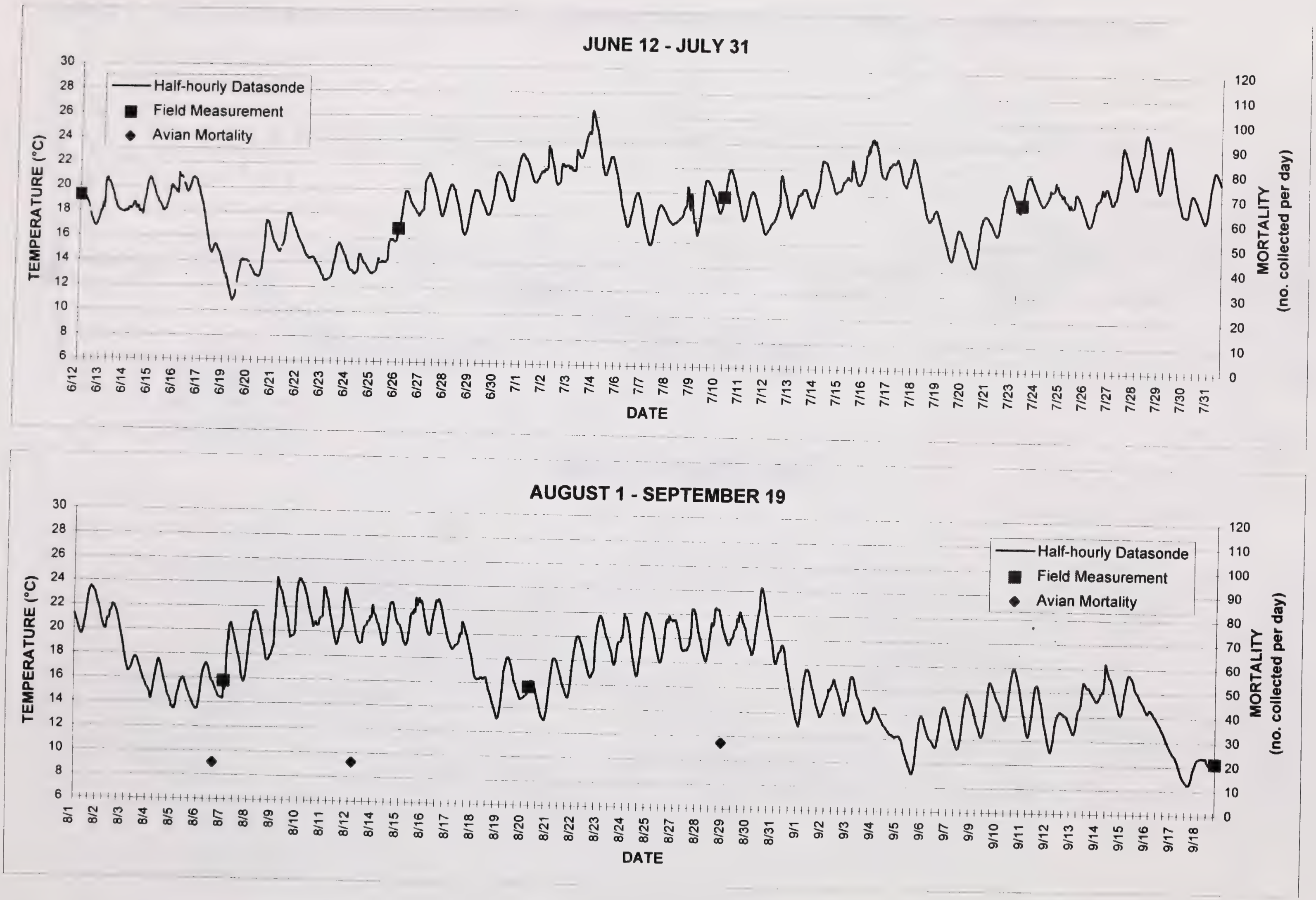


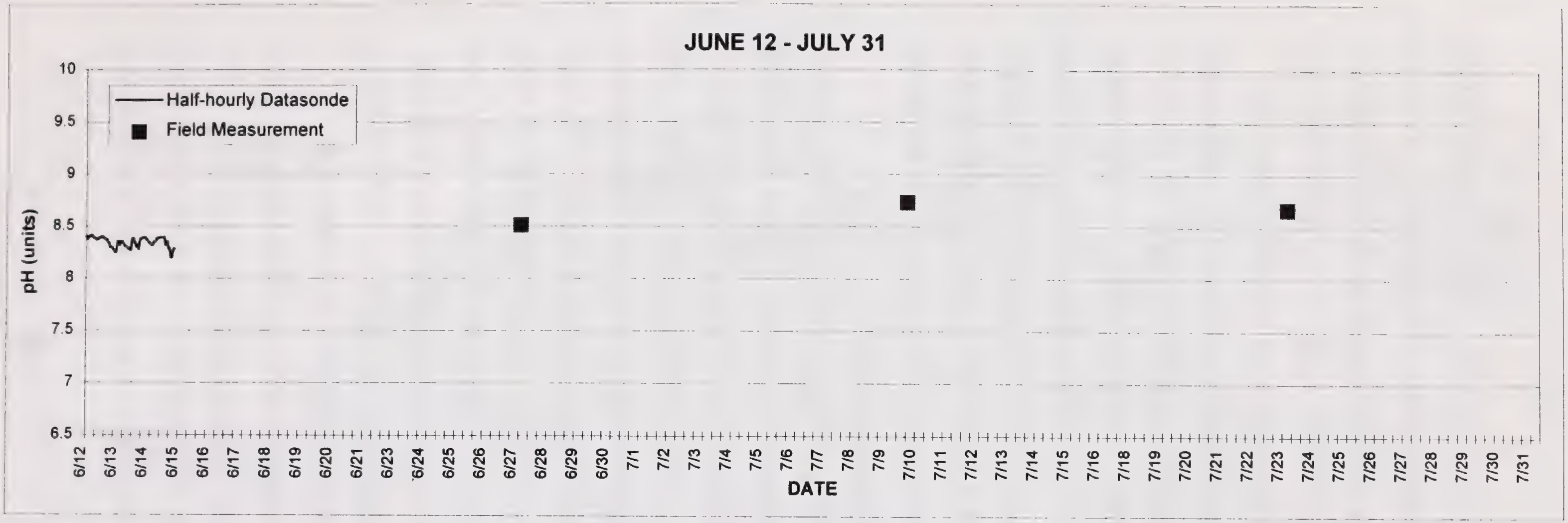
Figure 5. Temperature and avian mortality in Pakowki Lake northwest bay, June 12-September 19, 1996.

basin between August 7 and August 11, when about 100 bird carcasses per day were collected, and the mid-day field measurements prior to August 7 were also relatively high (Figure 2). Overnight minimum oxygen levels at the datasonde site in the central basin gradually declined to less than 2 mg/L between August 11-17 (Figure 2), when avian mortality appeared to increase in that basin, while the area west of the berm was well-oxygenated during the same time period (Figure 3).

Although oxygen data were not available for critical time periods in 1996, data that were collected suggest a possible link between algal production and the onset of avian botulism in Pakowki Lake. Because the lake is extremely shallow (mean depth of 1.2 m at an elevation of 856.0 m a.s.l.), the water column was well-aerated early in the summer, due to wind-induced turbulence. Algal biomass in both basins increased greatly after July 10, and peaked in early August, just before peak avian mortality was reported (Section 3.5, Figure 10). Increasing algal biomass may trigger the onset of avian botulism through nocturnal oxygen depletion, and by coating the lake bottom with organic material, thereby inducing low redox in sediments.

Water temperature above 20°C is considered necessary for the rapid production of lethal levels of toxin by *Clostridium botulinum* type C (Haagsma 1974), the most frequent cause of avian botulism. In the central basin, the onset of avian mortality was preceded by four days of continuous water temperature over 20°C (Figure 4). However, a period of similarly high temperatures from July 3-6 was not followed by bird mortality. Once initiated, avian mortality continued to occur even at declining temperatures. Temperature west of the berm was generally similar to that in the central basin (Figure 5). Water temperature actually declined to 10-17°C in mid-September, when peak avian mortality occurred. Haagsma (1974) reported that *Clostridium botulinum* type C toxin persisted with little change in concentration for nine months, including the summer months, in a wetland in the Netherlands. Therefore, toxins produced in Pakowki Lake could persist and accumulate over the summer, and contribute to the high avian mortality rates in the fall.

The ASWQG (8.5) for pH was generally exceeded in the central basin, as was the CCME guideline for the protection of aquatic life (9.0), and pH was higher in the central basin than west of the berm (Figure 6 and 7). This variable increased in the central basin, and remained above both guidelines, from August 20 to September 3. The CCME guideline is mainly designed to protect fish,



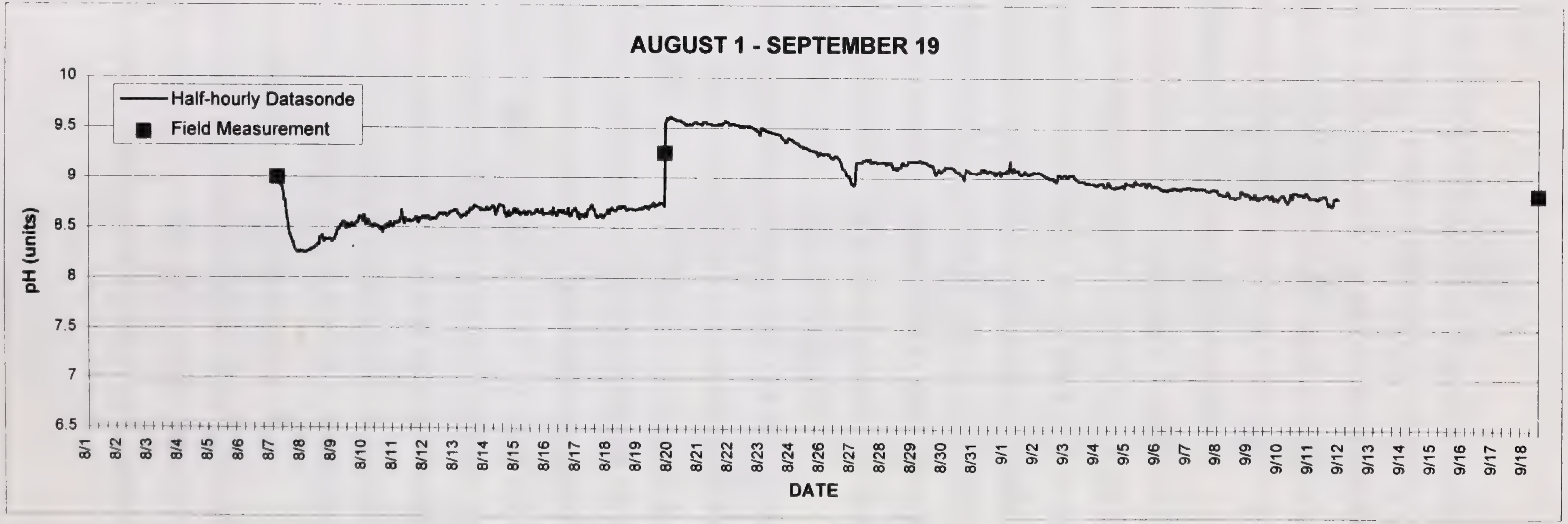


Figure 6. pH in Pakowki Lake central basin, June 12-September 19, 1996.

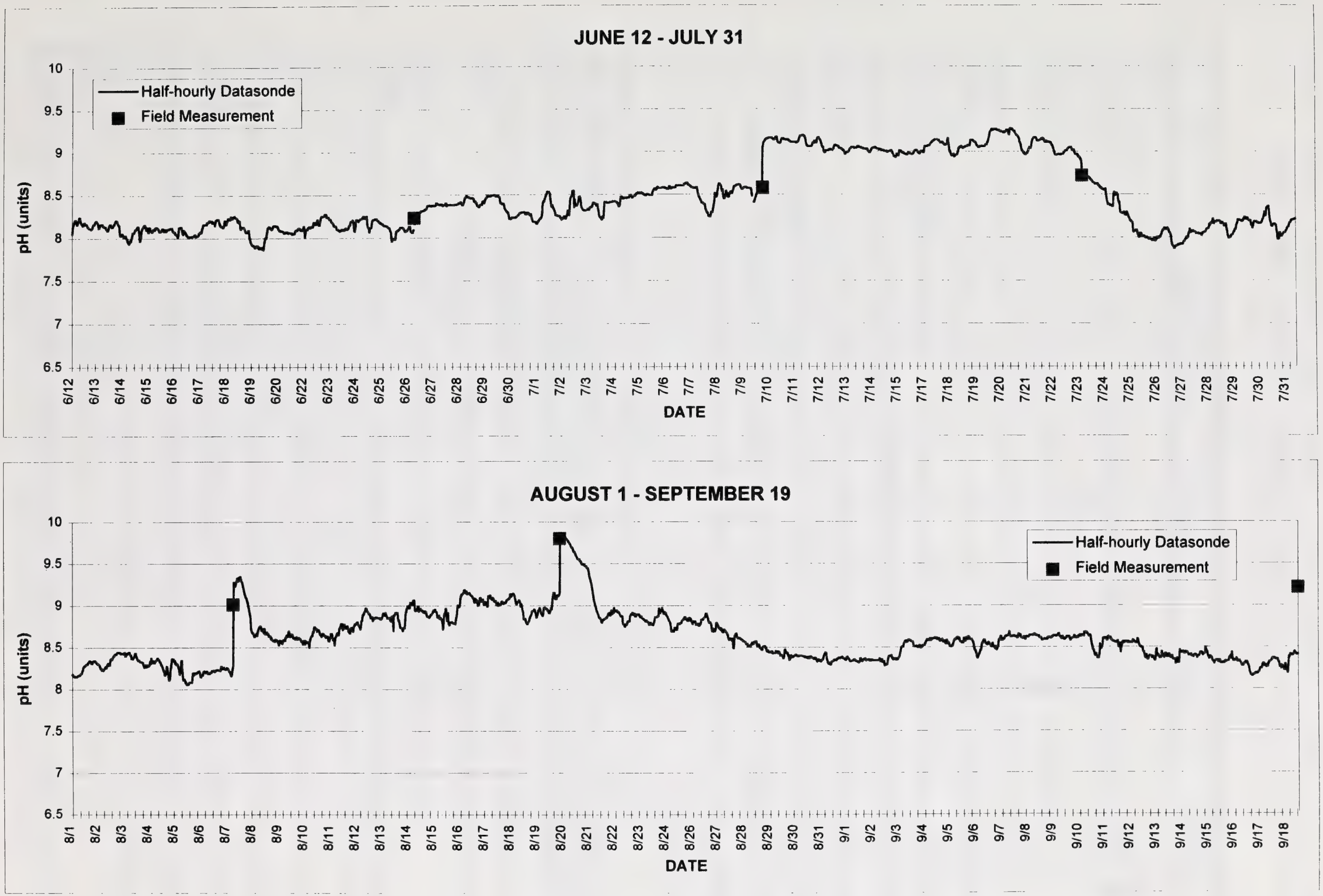


Figure 7. pH in Pakowki Lake northwest bay, June 12-September 19, 1996.

not wildlife, from the effects of high pH. As will be discussed in Section 3.4, a large increase in pH occurred at both datasonde sites on August 20, and this increase coincided with the collapse of the algal bloom.

3.3 DISSOLVED AND SUSPENDED SOLIDS, CONDUCTIVITY AND TURBIDITY

Pakowki Lake was very high in total dissolved solids (TDS) (median of 2110 mg/L, as total filtrable residue), and levels increased over the season with evaporation (Table 2, Appendix I). Late in the season, Pakowki Lake would be classified as "moderately saline" (TDS of 3001-10,000 mg/L) (CCME 1995). Sodium and sulphate in Pakowki Lake were quite high, and the concentration of most ions increased over the season with evaporation. Major ions in Pakowki Lake were in the ratio $SO_4^{2-}>Na^{+}>Mg^{2+}>Cl^{-}>Ca^{2+}>K^{+}$. This order of major ions is higher in chloride, and lower in potassium, than other moderately saline lakes in Alberta (Mitchell and Prepas 1990). Etzikom Coulee was much higher in TDS than Manyberries Creek (Table 2), but lower than Pakowki Lake.

Conductivity in the central basin increased steadily over the summer from 2490 µS/cm in May (field measurement with Hydrolab) to levels over 3800 µS/cm by September 15 (Figure 8). This increase in conductivity is probably caused by an increase in the concentration of dissolved salts due to evaporation. Conductivity was lower west of the berm than in the central basin, and declined west of the berm in late summer as flow resumed in Etzikom Coulee (Figure 9).

Carbonate levels in Pakowki Lake were much higher on August 20 than on other sampling days (Appendix I), presumably due to a shift in the carbonate:bicarbonate equilibrium caused by photosynthetically-induced changes in pH. A large increase in pH occurred at both datasonde sites on August 20 (Figure 6 and 7), and chlorophyll *a* levels declined greatly after this date (Figure 10). There were differences between datasondes in pH calibration, which sometimes resulted in a shift in the pH record when units were exchanged in the lake. This complicated interpretation of the pH data. However, the pH increase west of the berm on August 20 (Figure 7) began earlier in the day that the datasondes were changed.

These findings support the hypothesis of Murphy *et al.* (1996) that the collapse of cyanobacterial blooms is coincident with calcium carbonate precipitation. However, it should be

Table 2. Median concentration (mg/L, except as noted) of selected variables in two areas of Pakowki Lake, and in tributary streams, during May 28-October 10, 1996. Variables for which half the data (Appendix I) exceeded guidelines are indicated in bold.

VARIABLES	PAKOWKI L., CENTRAL BASIN	PAKOWKI L., WEST OF BERM	ETZIKOM COULEE AT SR 855	MANYBERRIES CK. S. OF ORION
Sample Type	composite	composite	grab	grab
Number of Samples	9	9	6	4
Nutrients				
Total Phosphorus	**0.793**[3]	**0.650**[3]	**0.261**[3]	**0.146**[3]
Total Dissolved Phosphorus	0.503	0.283	0.232	0.130
Total Kjeldahl Nitrogen	2.46	2.20	1.59	1.15
Ammonia	0.12	0.11	0.08	0.04
Nitrite/Nitrate	0.133	0.016	0.002	0.002
Total Nitrogen	2.77	2.30	1.60	1.15
Silica	12.80		6.50	7.73
Available N/Available P Ratio	0.50	0.25	0.15	0.43
Dissolved Organic Carbon	23.10	22.80	23.65	18.55
Miscellaneous				
Chlorophyll *a*	34.6	31.7		
Sulphide (n=3)	<0.005	<0.005		
Total phenols	<0.001			
Total Dissolved Solids (calc.)	**2211**[2]			
Total Dissolved Solids (meas.)	**2110**[2]		**1695**[2]	**1085**[2]
Non Filtrable Residue	74.0		4.35	2.35
Turbidity	120			
True Colour	40			
Alkalinity (Total)	424			
Alkalinity (PP)	25			
pH	**8.67**[3]			
Total Hardness	412			
Sodium Adsorption Ratio	**12.6**[2]			
Major Ions				
Sodium	**587**[2]			
Calcium	43.2			
Magnesium	69.4			
Potassium	21.2			
Chloride	67.5			
Sulphate	**1090**[2]			
Carbonate	30			
Bicarbonate	459			
Metals				
N	3			
Aluminum	**1.320**[1]			
Arsenic	**0.029**[3]			
Boron	0.150			
Cadmium	<0.0002			
Chromium	<0.002			
Copper	**0.018**[1]			
Iron	**2.530**[1]			
Lead	0.003			
Manganese	0.079			
Mercury, μg/L	<0.05			
Nickel	<0.0005			
Selenium	0.0002			
Vanadium	0.012			
Zinc	0.019			

Water Quality Guidelines:

[1] CWQG for the Protection of Freshwater Aquatic Life

[2] CWQG for Agricultural Uses

[3] AWQG

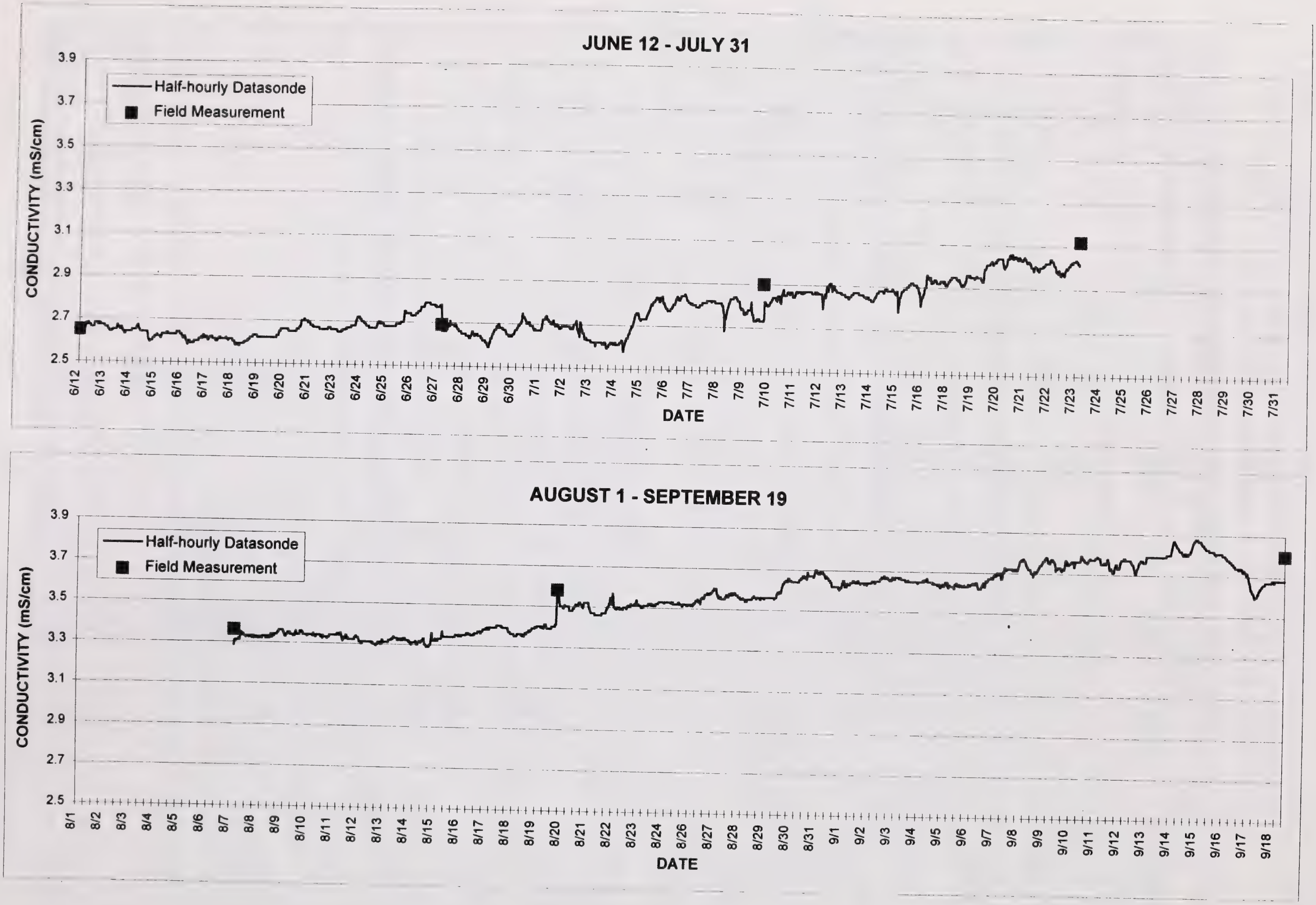
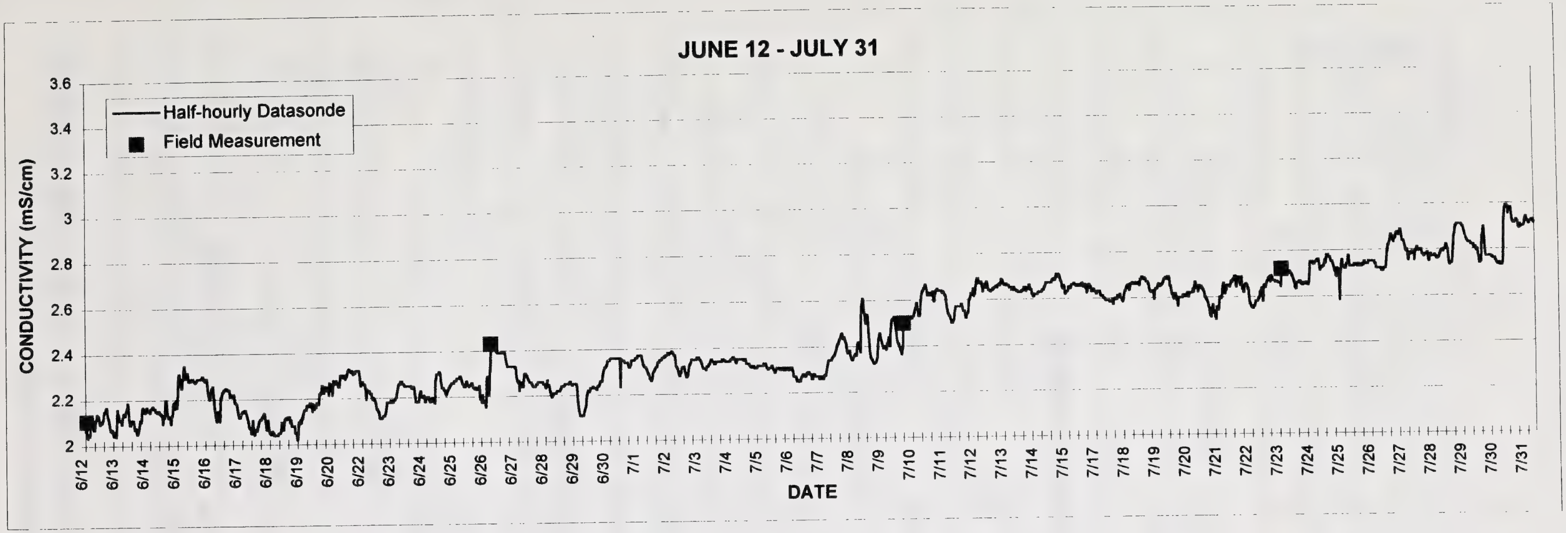


Figure 8. Conductivity in Pakowki Lake central basin, June 12-September 19, 1996.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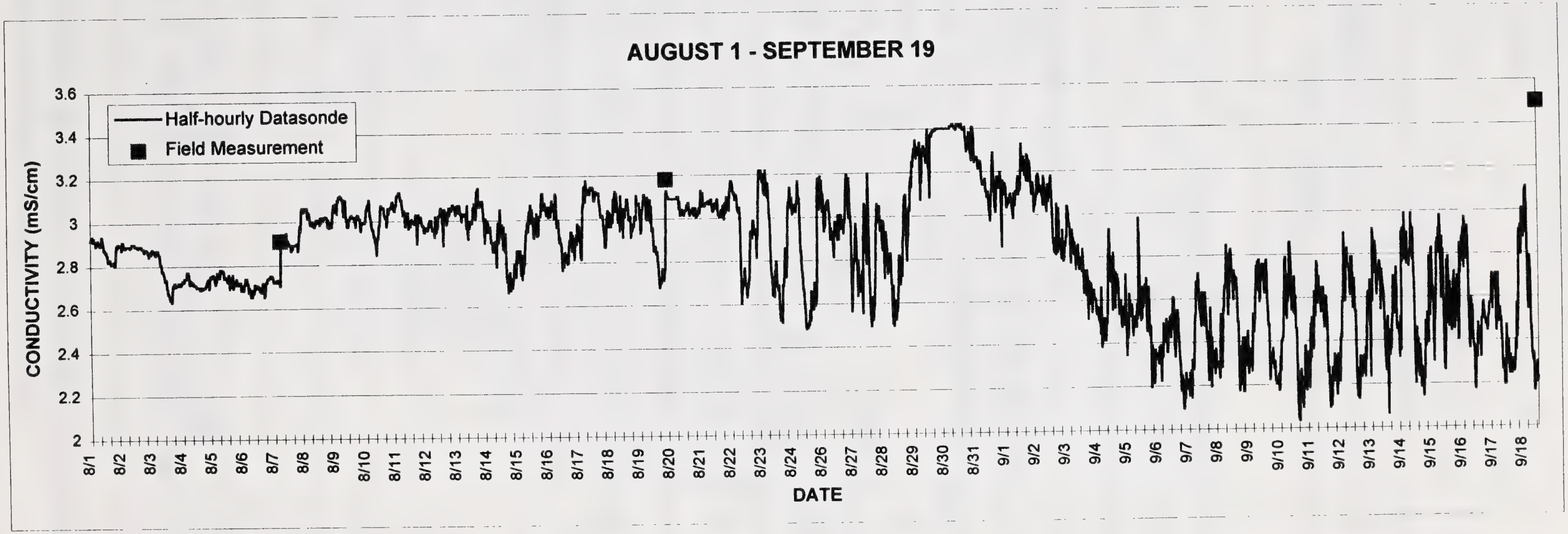
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Figure 9. Conductivity in Pakowki Lake northwest bay, June 12-September 19, 1996.

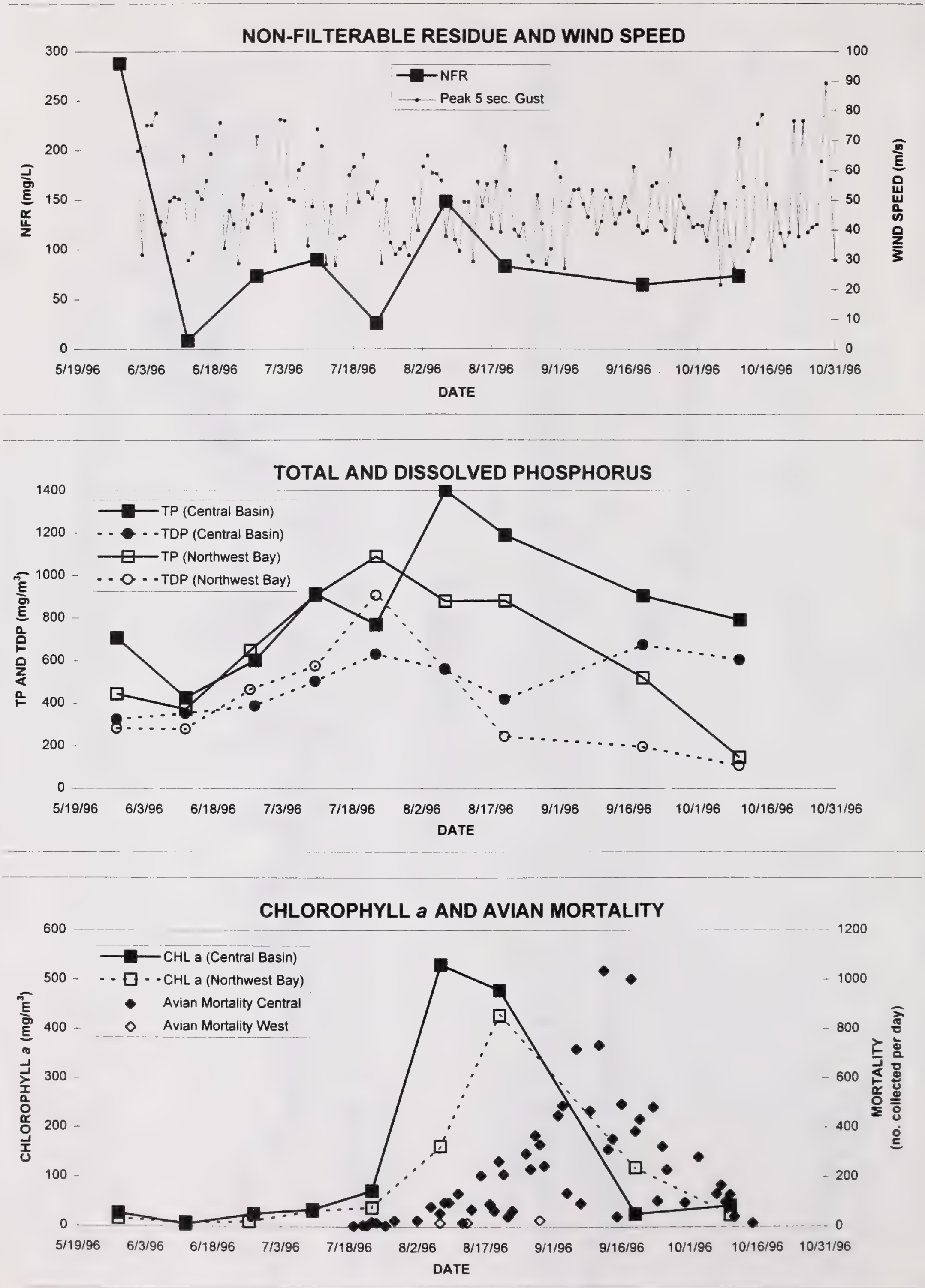


Figure 10. Comparison of NFR, wind speed, TP, TDP, CHL *a* and avian mortality in the central basin and northwest bay of Pakowki Lake, 1996.

noted that avian mortality in Pakowki Lake began about July 19, well before the increase in pH on August 20, and there was an increase in avian mortality even before the algal bloom collapsed (Figure 10). Therefore, these findings do not support the link between calcium carbonate and the onset of avian botulism proposed by Murphy *et al.* (1996), in which calcium carbonate precipitate induces reducing conditions in sediments, and triggers the onset of botulism.

TDS, sodium absorption ratio (SAR) and sodium in Pakowki Lake usually exceeded the ASWQG and CCME guidelines for the irrigation of sensitive crops (Appendix I), which include most vegetables, fruit and intolerant forage crops such as alfalfa, but was acceptable for tolerant crops, such as sugar beets or certain grains. Sulphate exceeded the CCME guideline for livestock watering after July 10 (Appendix I). Water withdrawn from Pakowki Lake in a proposed lake management program (e.g., dewatering of the lake, as evaluated in Wohl (1996)) would be subject to restrictions for agricultural use.

Pakowki Lake was far more turbid (median 120 NTU) than most Alberta lakes (average of 12 NTU for moderately saline lakes) (Mitchell and Prepas 1990). Furthermore, nonfiltrable residue (NFR) (a measure of suspended sediment and particulate organic material) was also extremely high (Table 2) most of the season. The technologist sampling Pakowki Lake (R. Walker) noted that a thick layer of sediment accumulated in samples left overnight. Fixed NFR, a measure of inorganic suspended sediment, was relatively high on the single date this was measured in Pakowki Lake (Appendix I). Secchi depth, a measure of light penetration, was always 0.3 m or less in Pakowki Lake, even when algal biomass (as chlorophyll *a*), was relatively low early in the summer (Figure 10).

These results suggest that non-algal turbidity is relatively high in Pakowki Lake. It appears that inorganic particulate material from the lake bed, such as fine silt and clay is continually re-suspended by wind-induced turbulence. Both Etzikom Coulee and Manyberries Creek had relatively low NFR during the sampling program, but there may have been higher suspended sediment loading from these streams earlier in the spring. The high turbidity and suspended sediment in this lake probably reduces light penetration and inhibits the growth of algae and macrophytes early in the season. The severe algal bloom that occurred in early August, was preceded by internal phosphorus release and by relatively low NFR on July 23 (Figure 10), perhaps due to a period of relatively calm conditions and settling of suspended sediment. Wind speed gradually declined over June and July at a nearby weather station (Figure 10).

3.4 ALGAL NUTRIENTS

3.4.1 Phosphorus

Pakowki Lake had extremely high levels of total phosphorus in 1996 (median of 0.793 mg/L) with a large portion of total dissolved phosphorus (TDP) (median 0.503 mg/L, Table 2; Figure 10). High TDP suggests that a large proportion of the phosphorus in this lake is biologically available to stimulate algal growth. The phosphorus levels measured in 1996 place this lake at the upper end of the hypertrophic range of algal productivity. Pakowki Lake is one of the most productive lakes in Alberta (Figure 11). Average (rather than median) total phosphorus for Pakowki Lake has been plotted in Figure 11 to allow comparisons with a wide range of other Alberta lakes.

Both total and dissolved phosphorus increased greatly in July and early August, during a period of relatively low stream flow and external phosphorus loading (Figure 10). This increase in phosphorus strongly suggests the internal release of phosphorus from sources within the lake. These sources would include phosphorus release from lake sediments. The phosphorus mass balance (Table 3) suggests that a total of 141,092 kg of total phosphorus were released to Pakowki Lake between June 13 and August 7, and the greatest release (78,495 kg) occurred just before the major algal bloom (Section 3.6) of the season. Internally released phosphorus comprised 89.5% of the total phosphorus loading to the lake during the sampling period (May 28-October 10, 1996) compared to only 8.6% from streams and diffuse runoff, and 1.9% from precipitation for the period February 20-October 10, 1996.

These results strongly suggest that Pakowki Lake phosphorus levels (and algal productivity) are strongly influenced by the internal loading of phosphorus. Since Pakowki Lake is a terminal basin, without the loss of phosphorus to an outflow, phosphorus continually accumulates in this basin from all external sources. The major stream loading to Pakowki Lake appears to be Etzikom Coulee, which supplied an annual load of 8035 kg of total phosphorus (59.2% of the annual stream and diffuse loading to Pakowki Lake), compared to 1813 kg for Manyberries Creek (13.4%), and 3720.1 kg (27.4%) for all other tributaries and diffuse runoff combined.

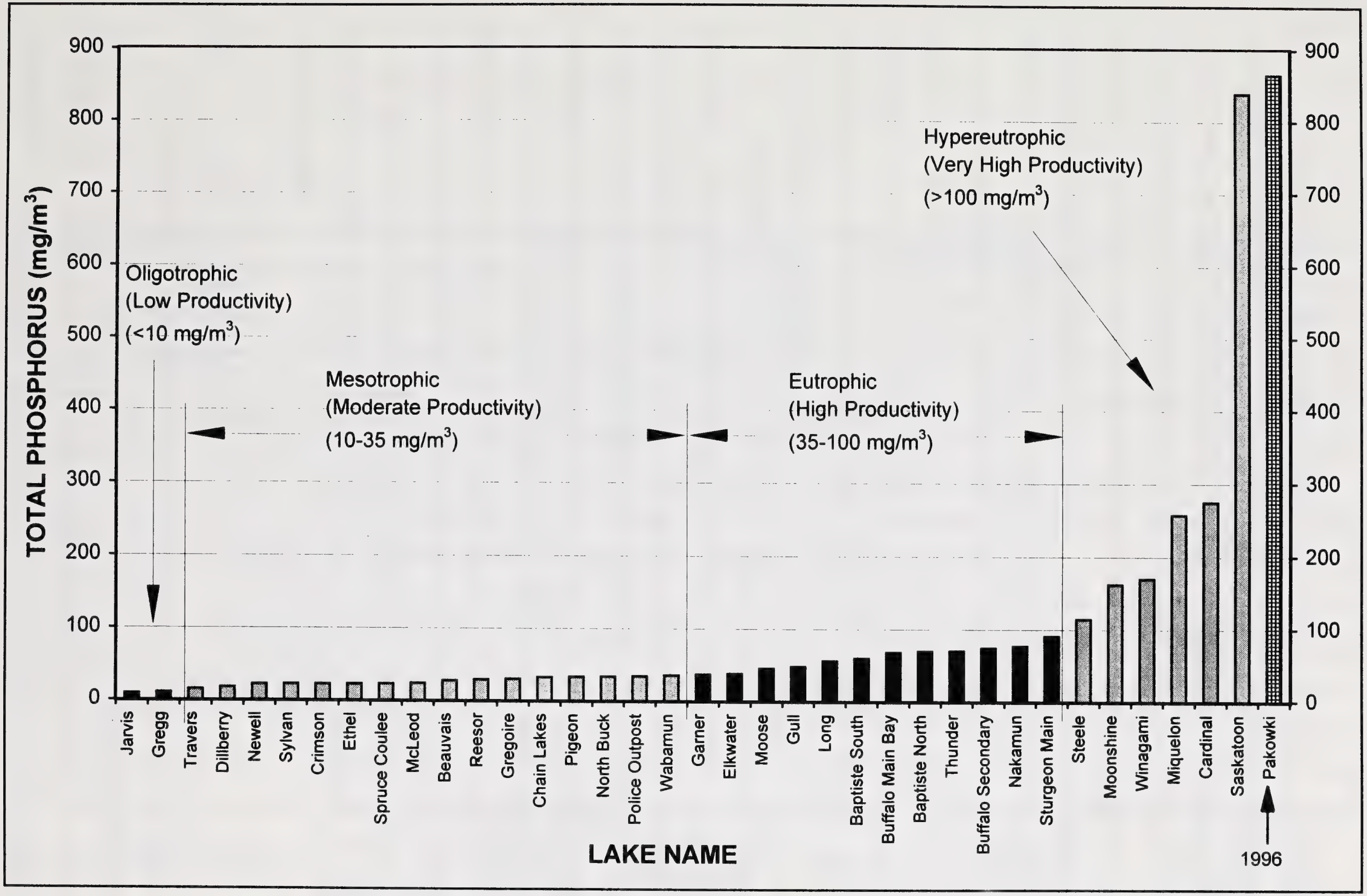


Figure 11. Approximate trophic categories for Alberta lakes based on average summer total phosphorus concentrations, 1983-1996.

Table 3. Summary of mass balance analysis for total phosphorus in Pakowki Lake for February 20-October 10, 1996.

INTERVAL END DATE	[TP] LAKE	LAKE VOLUME, m^3	ESTIMATED LAKE LEVEL, m	TP MASS IN STREAMS +DIFFUSE RUNOFF, kg	TP MASS, ATMOSPHERIC DEPOSITION, kg	TP MASS, LAKE, kg	CHANGE IN LAKE TP MASS BETWEEN INTERVALS, kg	NET INTERNAL TP MASS RELEASED (+) OR DEPOSITED (-), kg
19 Feb 1996								
27 May-96				10,379.1	1,156.0			
28-May-96	0.707	164,452,851	856.30		0.0	116,268.2		
12-Jun-96	0.427	158,911,199	856.25	159.9	54.6	67,855.1	-48,413.1	-48,627.6
27-Jun-96	0.602	149,429,530	856.17	88.6	1,259.0	89,956.6	22,101.5	20,753.9
10-Jul-96	0.913	144,791,200	856.13	359.6	34.8	132,194.4	42,237.8	41,843.4
23-Jul-96	0.770	133,105,683	856.02	154.7	12.1	102,491.4	-29,703.0	-29,869.8
7-Aug-96	1.400	129,355,813	855.99	10.2	101.6	181,098.1	78,606.8	78,494.9
20-Aug-96	1.190	124,242,082	855.93	26.0	18.8	147,848.1	-33,250.1	-33,294.9
19-Sep-96	0.906	116,878,429	855.85	938.4	246.4	105,891.9	-41,956.2	-43,141.0
10-Oct-96	0.793	119,878,000	855.88	1,451.4	54.9	95,063.3	-10,828.6	-12,334.9
Season Total				13,567.8	2,938.3		total release (+)	141,092.2
							total deposition (-)	-167,268.2
% of Total				8.61%	1.86%		total proportion of load	89.53% (+)

The relatively large phosphorus load from Etzikom Coulee is probably due to loadings from agricultural sites, municipal wastewater and other sources. Agricultural sources along Etzikom Coulee were not identified during the present study, but loadings from various sites should be measured if control of external loading is to be considered. The Town of Raymond is the only community that currently releases treated wastewater to Etzikom Coulee (A. Pentney, Environmental Regulatory Service, AEP; personal communication). The Town of Raymond typically releases about 240,000 m^3 of wastewater in spring and fall. If a total phosphorus concentration of 3 mg/L is assumed for this wastewater, this volume would contain 720 kg, which is only 9.0% of the estimated annual load from Etzikom Coulee to Pakowki Lake in 1996. After the spring of 1997, and until its population exceeds 4,000, the Town of Raymond will normally use a combination of storage and irrigation instead of direct discharge to Etzikom Coulee (A. Pentney, Environmental Regulatory Service, AEP; personal communication).

The mass balance analysis used certain assumptions about loadings from surface runoff and rainfall. To complete the analysis as soon as possible, *preliminary* WSC stream flow data were used for the estimation of stream loadings. These flow data are subject to change. Daily rainfall volumes in 1996 from the nearest available weather station, near Aden, were used on the assumption that these were typical of precipitation at Pakowki Lake, and rainfall chemistry measured near another hypertrophic lake (Eagle Lake) in southern Alberta was used. Stream and lake total phosphorus concentration were typically measured every other week. This infrequent stream sampling probably missed periods of higher phosphorus loading during storm events. Median stream phosphorus during the sampling period (May 28-October 10) was used to estimate the approximate loading during spring runoff. This may underestimate phosphorus loading during spring snowmelt.

3.4.2 Nitrogen and Reactive Silica

Pakowki Lake had relatively low nitrogen levels in 1996. Except for ammonia on one sampling date (Appendix I), the various forms of nitrogen were always less than water quality guidelines. The ratio of available N:available P (ammonia and nitrite/nitrate divided by TDP) was extremely low (median of 0.50). An N:P ratio of 7.0 is often used as the dividing line between nitrogen and phosphorus limitation in aquatic systems. The low ratio in Pakowki Lake strongly favours the growth of cyanobacteria (such as *Aphanizomenon flos-aquae*) which are able to fix dissolved nitrogen (N_2) and thereby proliferate in an otherwise nitrogen-limited lake.

Pakowki Lake and the sampled tributaries were always high in reactive silica (Table 2), which is necessary for the development of diatom communities. At extremely low levels the concentration of reactive silica can limit the growth of diatoms and thereby alter the species composition of the algal community (Wetzel 1983).

3.5 BIOLOGICAL VARIABLES

3.5.1 Phytoplankton Chlorophyll *a*

Chlorophyll *a* is often measured in lakes as an indicator of phytoplankton biomass. In this study, phytoplankton biomass was also directly estimated on monthly samples (Section 3.5.2). Chlorophyll *a* varied greatly in Pakowki Lake over the season (Figure 10). Early in the sampling period, chlorophyll *a* levels were relatively low both west of the berm, and in the central basin. However, chlorophyll *a* levels increased greatly after July 10 and peaked during an algal bloom in early August (this bloom is described in more detail in Section 3.5.2 and Appendix II). The peak chlorophyll *a* levels in Pakowki Lake in 1996 were higher than values measured in other lakes and reservoirs in Alberta (Figure 12), and elsewhere. Wetzel (1983) presents a maximum of 275 µg/L in a summary of peak chlorophyll *a* for lakes and reservoirs throughout the world. Based on summer average chlorophyll *a*, Pakowki Lake is near the top end of the hypertrophic range of algal productivity.

Under calm conditions, which rarely occurred, grab sampling could bias the chlorophyll *a* results from Pakowki Lake. Due to its shallow depth, Pakowki Lake was sampled using a composite of subsurface grab samples, whereas vertically-integrated euphotic zone composite samples were collected for the other lakes in Figure 11 and 12. Although the grab samples were collected well below the surface algal layer, there could have been a higher concentration of algal cells at that depth than deeper in the euphotic zone.

Peak chlorophyll *a* levels occurred just before a dramatic increase in the number of dead birds found in the central basin (Figure 10). However, large numbers of dead birds were found well

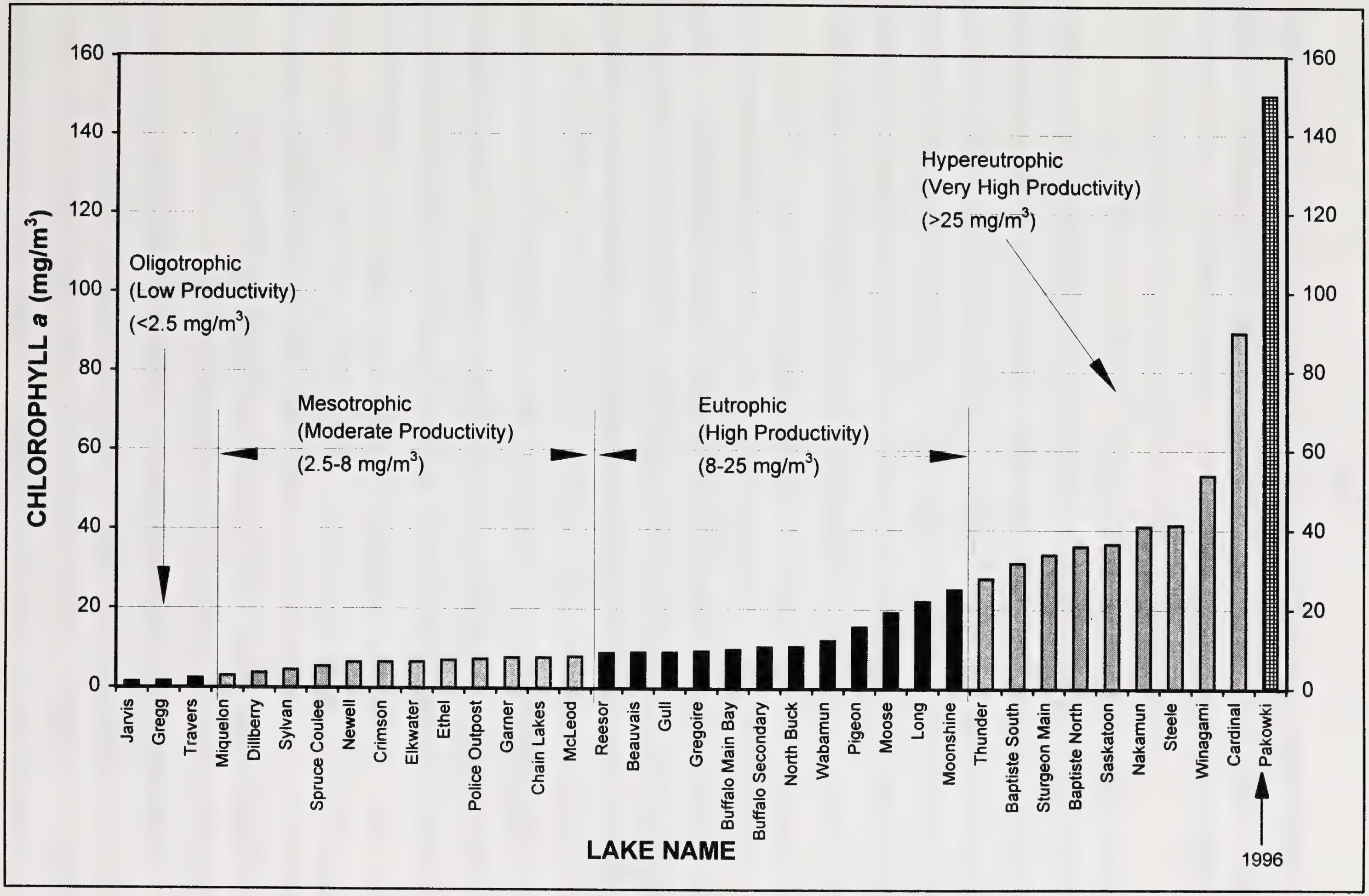


Figure 12. Approximate trophic categories for Alberta lakes based on average summer chlorophyll *a* concentrations, 1983-1996.

after the bloom had collapsed. This suggests that if mortality from avian botulism is influenced by algal blooms, some residual factor (e.g., stable cyanobacterial toxins) continues to influence mortality rates after the blooms subside.

3.5.2 Algal Species Composition and Biomass

The August algal bloom in both areas sampled at Pakowki Lake was dominated by the cyanobacterium *Aphanizomenonflos-aquae* (Appendix II). The bloom in the central basin contained nearly 13 times more biomass of this species on August 7 than the area west of the berm (Appendix II, Figure 1). Since taxonomic samples were only collected on a monthly schedule, it is not known whether *Aphanizomenon* was abundant west of the berm when chlorophyll *a* levels peaked there on August 20 (Figure 10). *Aphanizomenon* and other species of cyanobacteria were relatively uncommon in the other sampling months, compared to green algae and diatoms.

Aside from *Pediastrum*, which has an unusually large biovolume (but was <4% of total cell numbers), green algae were a small portion of the algal biomass in Pakowki Lake. Diatoms were a larger part of the total algal biomass than green algae, and bloomed mainly in September-October. Diatom biomass was much higher west of the berm than in the central basin at that time.

3.5.3 Cyanobacterial Toxins

Aphanizomenon flos-aquae can produce various neurotoxins, including anatoxin-a (Rapala *et al.* 1993), saxitoxin and neosaxitoxin, and several toxins not yet identified (Gorham and Carmichael 1988). Anatoxin-a was not detected in any of the algal samples from Pakowki Lake in 1996 (Appendix III). The analyst noted that some of the samples contained large amounts of dried zooplankton but relatively little algal biomass, which would reduce the sensitivity of the neurotoxin analysis on these samples (Figure 10). However, these results suggest there was little or no anatoxin-a produced during the main algal bloom in Pakowki Lake.

Because toxins other than anatoxin-a may be produced by *Aphanizomenon flos-aquae*, bioassays involving the interperitoneal injection of algal extract into mice were also conducted at the University of Saskatchewan. The full results are not yet available, but preliminary results indicate that a potent neurotoxin was present on September 19, 1996. This was the sampling date during the period of peak avian mortality at Pakowki Lake, but after the algal bloom collapsed in late August (Figure 10). Furthermore, the test mice were not protected by botulinal antitoxin (T. Bollinger, Dept. of Veterinary Pathology, Univ. of Saskatchewan, personal communication), which indicates that the toxin was not produced by *Clostridium botulinum* type C.

Cyanobacterial toxin production may have been delayed by changes in water temperature. Rapala *et al.* (1993) found that production of anatoxin-a by *Aphanizomenon flos-aquae* was highest late in the growth cycle at 15°C, and was inhibited at high temperatures (≥ 25°C). At Pakowki Lake, water temperature in the central basin was generally 12-17°C in early September, during peak avian mortality, and did not exceed 25°C at any time (Figure 4). The cyanobacterial neurotoxin produced in Pakowki Lake in 1996 has not been identified, and it is not known whether it was present in amounts that could cause avian mortality through ingestion.

3.6 METALS

Extractable aluminum, iron, copper and lead occasionally exceeded the CCME guidelines for the protection of aquatic life (Appendix I). Aluminum and iron levels were especially high, compared to concentrations usually measured in lakes. Since there were very high levels of suspended sediment in this lake, values exceeding guidelines probably reflect particulate metals which would have lower toxicity than dissolved metals. The guidelines for these metals were developed primarily for the protection of fish and other aquatic organisms, rather than waterfowl. There does not appear to be a direct link between the levels of these metals in Pakowki Lake and avian mortality.

3.7 HERBICIDES AND PESTICIDES

Low levels of 2,4-D (0.096 µg/L) were detected in the single lake sample that was collected on October 10, 1996, but otherwise none of the 31 herbicides and pesticides included in the three scans (sample scan in Appendix IV) from the central basin and two tributaries were above the method detection limits. The single detected herbicide, 2,4-D, was well below the CCME guideline for the most sensitive use (4.0 µg/L for the protection of freshwater aquatic life). There is, therefore, no evidence from the October 1996 samples that toxic effects due to herbicides and pesticides contributed to avian mortality in Pakowki Lake.

Higher concentrations of herbicides and pesticides could occur in Pakowki Lake at other times of the year, since areas within the Pakowki watershed have the highest sales in Alberta of 2,4-D (all isomers), carbofuran and dicamba (Cotton and Byrtus 1995). However, it should be noted that in 1996 only 6.6% of the annual flow from Etzikom Coulee, and 2.7% of the Manyberries Creek flow entered Pakowki Lake during June to August, when pesticide and herbicide usage in the watershed would have been high.

3.8 AVIAN BOTULISM MODEL FOR PAKOWKI LAKE

The results of the 1996 water quality study strongly suggest a link between algal production in Pakowki Lake, and the onset and continuation of an outbreak of avian botulism. Potential relationships between physical, chemical and biological conditions in the lake in 1996, and mortality from avian botulism, are summarized in Figure 13. The various components in this flow diagram have been arranged in chronological sequence according to key events in the 1996 outbreak (e.g., first avian mortality on July 19), and suspected cause-effect relationships are indicated by arrows between components. To simplify the diagram, some components and linkages have been presented a single time. For example, relatively high water temperatures occurred during various time periods in 1996, but only the two time periods before the onset of avian mortality have been shown in Figure 13.

Environmental factors that initiate and maintain an avian botulism outbreak could function as follows. Early in the summer, high water temperatures in shallow Pakowki Lake allow the warming of bottom sediments, the development of low redox conditions below the sediment water interface, and the subsequent release of sediment-bound phosphorus. This could occur even though water column conditions remains oxic (Marsden 1989). The mass balance for Pakowki Lake (Table 3) determined that large amounts of total phosphorus were released during two time intervals (June 13-July 10; July 24-August 6) which included periods when water temperature greatly exceeded 17°C.

As a result of phosphorus loading from sediments, algal production greatly increased after July 10, which in turn caused fluctuations in dissolved oxygen, leading to nocturnal depletion and

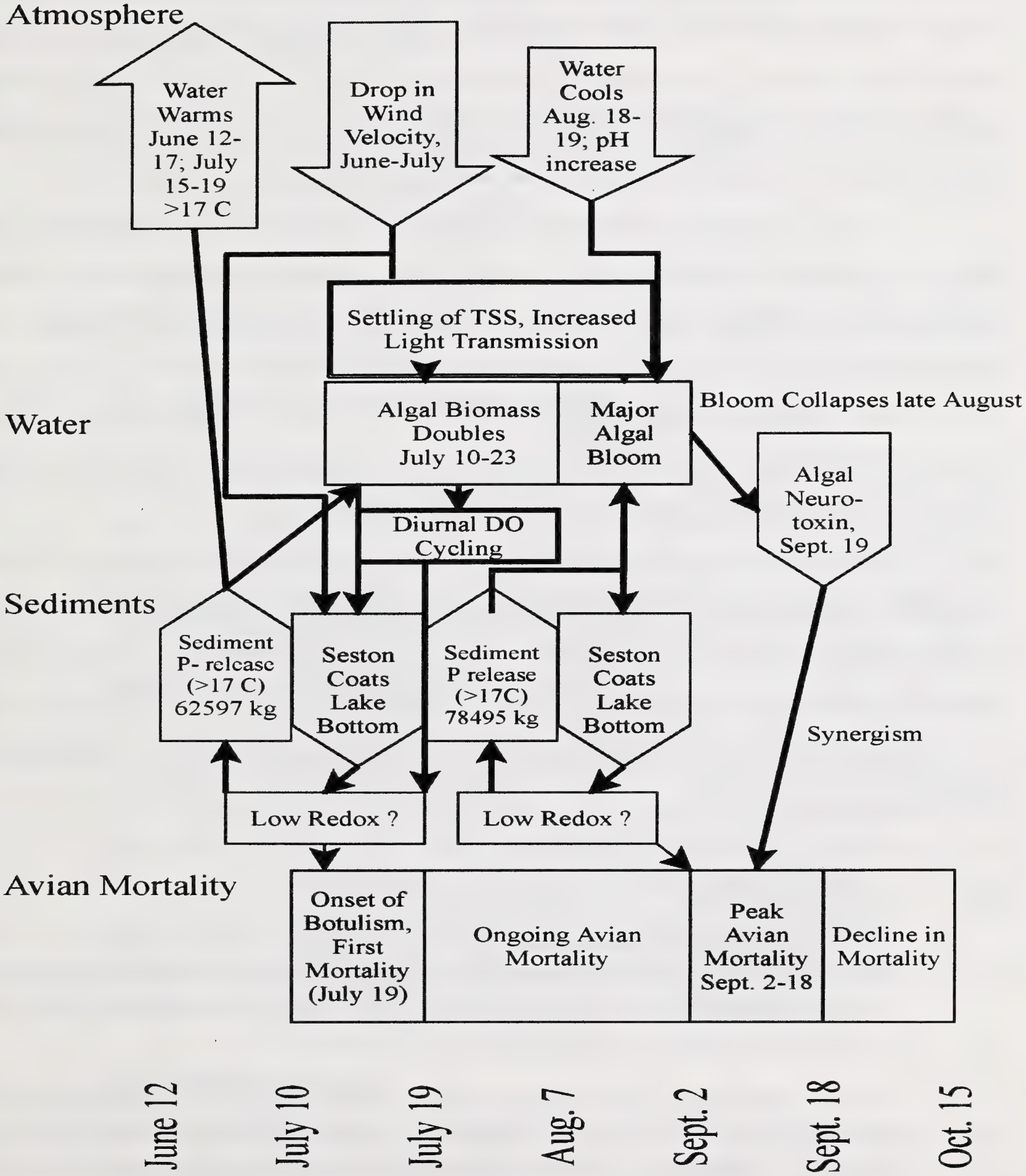

Atmosphere
Water Warms June 12-17; July 15-19 >17 C
Drop in Wind Velocity, June-July
Water Cools Aug. 18-19; pH increase
Settling of TSS, Increased Light Transmission
Water
Algal Biomass Doubles July 10-23
Major Algal Bloom
Bloom Collapses late August
Algal Neuro-toxin, Sept. 19
Diurnal DO Cycling
Sediments
Sediment P- release (>17 C) 62597 kg
Seston Coats Lake Bottom
Sediment P release (>17C) 78495 kg
Seston Coats Lake Bottom
Synergism
Low Redox ?
Low Redox ?
Avian Mortality
Onset of Botulism, First Mortality (July 19)
Ongoing Avian Mortality
Peak Avian Mortality Sept. 2-18
Decline in Mortality
June 12
July 10
July 19
Aug. 7
Sept. 2
Sept. 18
Oct. 15

Figure 13. Physical, chemical and biological factors that may contribute to the onset and continuation of an outbreak of avian botulism in Pakowki Lake.

increased amounts of algal biomass in the water column. During the same time period, a decrease in wind velocity (Figure 10) occurred and resulted in the deposition of suspended sediment and increased water clarity. The combination of nocturnal oxygen depletion and deposition of large amounts of seston (inorganic and organic particulate material) from the water column onto lake sediments may induce low redox in sediments, and trigger the onset of avian botulism.

Increased water clarity, high water temperature and internal phosphorus loading would favour the continuation of the algal bloom, and continued deposition of seston onto warm lake sediments, which would maintain low redox favourable for *Clostridium botulinum*. The algal bloom continued until a sudden drop in water temperature, and possibly calcite precipitation, during August 18-20. Avian mortality increased greatly after the major algal bloom in August, perhaps enhanced as a result of persistent algal toxins, and peaked about September 2-18. Peak levels of an algal neurotoxin were detected in algal material collected September 19.

The proposed relationship between algal productivity and avian botulism in Pakowki Lake must be confirmed by additional sampling, and further work is required to understand key elements of the model. For example, it has been postulated that increasing algal production and deposition of seston induced low redox in warm sediments and triggered the onset of avian botulism in Pakowki Lake. However, no valid water or sediment redox data were collected in Pakowki Lake in 1996.

4.0 CONCLUSIONS

1. Based on the number of dead birds (per km^2) recovered by the clean-up crews, avian mortality rates were lower in the impounded area west of the berm on the northwest arm of Pakowki Lake, than in the central basin in 1996. The onset and period of peak avian mortality in Pakowki Lake were similar in 1995 and 1996.

2. The two datasonde sites in Pakowki Lake (west of the berm, central basin) were well-oxygenated during the early part of the summer, as would be expected in a shallow lake exposed to high winds. Pronounced diurnal fluctuation in dissolved oxygen, caused by increasing algal production, began during the onset of avian botulism.

3. Water temperature exceeded 20°C (the level considered necessary for the rapid formation of lethal levels of toxin by *Clostridium botulinum* type C) for four days prior to the onset of avian mortality. However, similar water temperatures earlier in July were

not associated with mortality. The area west of the berm was slightly warmer than the central basin. Avian mortality increased through the summer even though water temperatures declined.

4. Dissolved solids, pH, SAR, sodium, sulphate, phenols and various metals in Pakowki Lake exceeded water quality guidelines for agricultural use or for the protection of aquatic organisms. However, there is no evidence that high levels of these substances increased the incidence of avian mortality.

5. Mean phosphorus and chlorophyll *a* levels in Pakowki Lake place this lake at the upper end of the hypertrophic range of algal productivity. It is among the most productive lakes in Alberta. Non-algal turbidity due to inorganic suspended sediment is extremely high in Pakowki Lake, compared to other lakes in Alberta. High turbidity may have a strong influence on the timing of algal blooms.

6. A preliminary mass balance analysis suggests that about 89.5% of the total phosphorus in Pakowki Lake is released from internal sources, compared to about 8.6% from tributaries and diffuse runoff and 1.9% from precipitation. The 1996 results suggest about 59% of the tributary phosphorus loading is from Etzikom Coulee, 13% from Manyberries Creek and 27% from four ungauged tributaries. Any lake management plan to reduce algal productivity in this lake must address the large internal loading of phosphorus in Pakowki Lake. The plan should also include a coordinated program to reduce phosphorus loading from surface runoff.

7. A severe algal bloom in Pakowki Lake occurred shortly before a large increase in the apparent rate of avian mortality in the central basin. This algal bloom consisted mainly of the cyanobacterium *Aphanizomenon flos-aquae*, which is capable of producing potent neurotoxins. *Aphanizomenon* was far less abundant west of the berm than in the central basin. A potent neurotoxin, other than *Clostridium botulinum* type C toxin, was detected in algal material collected in the central basin on September 19, when peak levels of avian mortality occurred. A link between algal productivity and the onset and maintenance of botulism outbreaks is suspected, but must be confirmed by additional sampling.

5.0 RECOMMENDATIONS

1. **Cyanobacterial Toxins.** Water quality data from the 1996 sampling program strongly suggest that cyanobacterial toxins contributed to avian mortality at Pakowki Lake. This important finding should be verified by an additional year of sampling. The 1997 sampling program should include the following elements designed to clarify the link between algal toxins and avian mortality in Pakowki Lake:

   (a) sampling of all the variables and sites sampled in the 1996 program;

(b) routine measurement of fixed nonfilterable residue, to determine the levels of inorganic suspended sediment. The high levels of turbidity in Pakowki Lake may be caused by the resuspension of inorganic sediment;

(c) continuous measurement of turbidity, or another measure of light transmission, to determine the role of water transparency in the initiation of algal blooms;

(d) routine measurement of total and dissolved metals to establish whether any metals are at levels that can cause toxic effects on aquatic organisms;

(e) sampling of algal material and analysis to identify the cyanobacterial toxin(s) produced in Pakowki Lake;

(f) residue analysis or histopathology on birds to determine the degree to which this toxin causes avian mortality;

(g) sampling of algal toxins during peak avian mortality, along transects that includes the central basin datasonde site, eastern shoreline areas and the area (Harty's Island) where avian mortality began in 1995 and 1996.

2. **Management Options.** To date, carcass cleanup is the only effective method that has been identified to reduce the incidence of avian mortality in Pakowki Lake during an outbreak of avian botulism. If the link between cyanobacterial toxins and avian mortality is firmly established, then reducing algal productivity could reduce avian mortality. The preliminary phosphorus budget for Pakowki Lake indicates that about 90% of the annual phosphorus loading is from internal sources. Therefore, in-lake treatment to reduce internal phosphorus loading could be an option.

Further work is required to determine which methods of in-lake treatment would be appropriate and cost effective. Due to the large size of Pakowki Lake, most of the conventional methods to reduce internal loading (e.g., dredging, chemical treatment) would be extremely expensive. Sampling to improve the accuracy of the phosphorus budget is also required. The 1997 program should include the following components to improve the phosphorus budget and clarify management options:

(a) Automated sampling of Etzikom Coulee and Manyberries Creek during the early spring in 1997, to determine tributary phosphorus loading prior to lake sampling. During 1996, only 23.2% of the annual inflow from Etzikom Coulee, and 2.8% from Manyberries Creek, occurred during the May 28-October 10 sampling program;

(b) Automated recording of lake level in 1997, to improve the precision of mass balance calculations;

(c) Land use surveys in 1997, and sampling in subsequent years, to identify agricultural sites and other sources that contribute the bulk of the nutrient loading along tributaries to Pakowki Lake;

(d) Sediment core sampling to determine the characteristics and phosphorus content of lake sediments in Pakowki Lake. From informal observations in 1996, it appeared that much of the lake bottom had a firm substrate with a relatively thin layer of fine sediment. If this fine sediment is the major source of internal phosphorus loading, in-lake treatment to remove this fine sediment could be an option;

(e) Sediment core incubation studies in 1997, to determine whether sediments would continue to release large amounts of phosphorus after a reduction in external loading, flushing of the lake or removal of a layer of fine sediments;

(f) Studies to determine the size and characteristics of the area near Harty's Island where avian botulism began in mid-July in 1995 and 1996. This work would include the installation of an additional datasonde for the month of July near Harty's Island to determine temperature, oxygen and redox. If botulism begins in a relatively small area of sediments with low redox, management options could include chemical treatment of sediments with an oxidizing agent;

(g) Mapping of the macrophyte distribution and species composition, perhaps by low level, infrared aerial photography. Wetland treatment of inflow phosphorus loading, involving the enhancement of macrophyte communities, has been proposed as a method of controlling cyanobacterial blooms in Pakowki Lake. It would be useful to first estimate the current rate and efficiency of phosphorus uptake by macrophytes, and determine what proportion.of the internal load could be removed by wetland treatment.

6.0 LITERATURE CITED

Alberta Environmental Protection. 1993*a*. Water quality sampling methods for surface waters. Surface Water Monitoring Branch, Technical Services and Monitoring Division. 67 p.

Alberta Environmental Protection. 1993*b*. Alberta ambient surface water quality interim guidelines. Environmental Protection and Enhancement Act.

Bell, J.F., G.W. Sciple, and A.A. Hubert. 1955. A microenvironment concept of the epizoology of avian botulism. Journal of Wildlife Management 19: 352-357.

CCME. 1995. Canadian water quality guidelines. Task force on water quality guidelines of the Canadian Council of Ministers of the Environment.

Cotton, M.M. and G. Byrtus. 1995. Pesticide sales trends in Alberta. Appendix A2. Phase 2. Selection of soil landscape units and study design considerations for the surface water quality monitoring program. Prepared for the CAESA Water Quality Monitoring Committee. 66 p.

Gorham, P.R. and W.W. Carmichael. 1988. Hazards of freshwater blue-green algae (cyanobacteria). Chapter 16., p. 403-431. Lembi, C.A. and J.R. Waaland. eds. Algae and human affairs. Cambridge University Press.

Haagsma, J. 1974. Etiology and epidemiology of botulism in water-fowl in the Netherlands. Tijdschr. Diergeneesk. 99(8): 434-442.

Marsden, M.W. 1989. Lake restoration by reducing external phosphorus loading: the influence of sediment phosphorus release. Freshwater Biology 21: 139-162.

Mitchell, P. and E. Prepas. 1990. Atlas of Alberta lakes. University of Alberta Press, Edmonton. 675 p.

Munro, J.A. 1927. The waterfowl sickness at Lake Newell, Alberta, 1925-1926. Can. Field Nat. 41: 77-84.

Murphy, T.P., A. Lawson, J. Corsini, I. Gray, D.G. Rancourt. 1996. Whiteshell Lake. Biogeochemical study of 1996 botulism outbreak. National Water Research Institute, Environment Canada, Burlington. 49 p.

Neraasen, T. 1997. Agency perspectives on botulism control and research needs. Ducks Unlimited Canada. Paper presented at Avian Botulism Workshop, Jan. 23, 1997 at the Canadian Cooperative Wildlife Health Centre, University of Saskatchewan, Saskatoon. (presented by G. Brewster).

Pybus, M.J. and D. Eslinger. 1996. Waterfowl disease at Pakowki Lake. A cooperative cleanup operation involving: Alberta Fish and Wildlife, Ducks Unlimited Canada, Environment Canada. Alberta Fish and Wildlife Services, Alberta Environmental Protection, Edmonton. 30 p.

Rapala, J., K. Sivonen, R. Luukainen and S. I. Niemelä. 1993. Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena*-strains - a laboratory study. Journal of Applied Phycology 5: 581-591.

Rocke, T.E. 1997. Recent and proposed research in the United States. U.S. National Wildlife Research Center. Paper presented at Avian Botulism Workshop, Jan. 23, 1997 at the Canadian Cooperative Wildlife Health Centre, University of Saskatchewan, Saskatoon. (manuscript in preparation).

Rosen, M.R. 1971. Botulism. *In* Chapter 12. Davis, J.W., Anderson, R.C., Karstad, L. and D.O. Trainer. Infectious and parasitic diseases of wild birds. Iowa State University Press, Ames, Iowa.

Shaw, J. 1996. Draft. Alberta water quality guideline for the protection of aquatic life. Copper. Environmental Criteria Branch, Alberta Environmental Protection.

Wetzel, R.G. 1983. Limnology. Second Edition. Saunders College Publishing. 767 p.

Wohl, J. 1996. Pakowki Lake, Alberta. A preliminary investigation of water management options for the control of avian botulism. Submitted to the Pakowki Lake Working Group by Ducks Unlimited, Canada, Edmonton. 23 p., Appendices A-D.

Appendix I. Water chemistry of composite samples from two basins of Pakowki Lake and two tributaries in 1996. Variables exceeding guidelines are indicated in bold.

PAKOWKI LAKE, CENTRAL BASIN									
VARIABLES	**May 28**	**June 12**	**June 27**	**July 10**	**July 23**	**August 7**	**August 20**	**September 19**	**October 10**
TP (Chemex)	**0.707**[3]	**0.427**[3]	**0.602**[3]	**0.913**[3]	**0.770**[3]	**1.400**[3]	**1.190**[3]	**0.906**[3]	**0.793**[3]
TP (AEP)	0.728	0.410	0.567	0.719	0.750	1.240	0.883	0.825	
TDP	0.325	0.352	0.388	0.503	0.631	0.560	0.419	0.675	0.604
TDP (AEP)	0.190	0.310	0.000	0.361	0.440	0.579	0.558	0.384	0.664
TKN	2.46	2.29	2.3	1.91	2.6	6.8	8.75	2.05	2.97
Ammonia	0.12	0.5	0.22	0.09	0.06	0.11	0.06	***0.96***[1]	0.22
Nitrite/Nitrate	0.042	0.239	0.469	0.07	0.133	0.005	0.003	0.757	0.842
TN	2.5	2.53	2.77	1.98	2.73	6.8	8.75	2.81	3.81
Silica	8.2	9.7	10.9	12.8	4.25	16.4	19.8	18	14.6
DOC	18.4	19.8	23.1	21.4	23.1	24.3	29.4	30.4	33.2
Chloro *a*	26.9	4.7	25.6	34.6	72.9	530.1	478.6	26.0	42.0
Secchi Depth, m	0.1	0.3	0.1	0.1	0.1	0.2	0.2	0.2	0.1
Sulphide							<0.005	<0.005	<0.005
Total Phenols	<0.001	<0.001	**0.002**[1]	<0.001	<0.001	**0.017**[1]	<0.001	<0.001	<0.001
TDS (calc.)	**1610**[2]	**1780**[2]	**1900**[2]	**1890**[2]	**2211**[2]	**2309**[2]	**2513**[2]	**2802**[2]	**2863**[2]
TDS (meas.)	**1450**[2]	**2000**[2]	**2050**[2]	**2110**[2]	**2140**[2]	**2097**[2]	**2220**[2]	**3210**[2]	**3158**[2]
NFR	288	8.7	74	90	26	148	83	65	74
NFR, fixed									55.0
Turbidity	380	62	141	176	103	100	120	140	120
True Colour	10	80	30	50	40	40	40	70	200
Alkalinity (tot.)	322	351	382	424	454	507	531	578	372
Alkalinity (PP)	6.6	9.6	18.3	23.6	29.8	45.2	61.7	30.5	25
pH	8.35	8.41	8.48	8.67	8.73	8.9	9.02	8.67	8.63
Total Hardness	316	328	338	347	412	424	417	489	519
SAR	**10.77**[2]	**10.93**[2]	**12.40**[2]	**11.76**[2]	**12.58**[2]	**13.40**[2]	**14.76**[2]	**15.98**[2]	**15.13**[2]
Sodium	**440**[2]	**455**[2]	**524**[2]	**503**[2]	**587**[2]	**634**[2]	**693**[2]	**812**[2]	**792**[2]
Calcium	38.7	41.1	38.7	43.2	50.7	51.1	39.4	49.4	57.5
Magnesium	53.3	54.8	58.6	58	69.4	71.9	77.5	88.8	91.1
Potassium	15.7	16	17.7	17.1	21.2	21.9	22.9	25.8	24.8
Chloride	53	61	60.3	63.8	67.5	80	86	99.3	95.7
Sulphate	811	932	969	950	**1090**[2]	**1140**[2]	**1270**[2]	**1370**[2]	**1570**[2]
Carbonate	7.9	11.5	21.9	28.3	35.7	54.3	74.1	36.6	30
Bicarbonate	377	404	421	459	481	508	497	630	393
Aluminum	**1.66**[1]					**0.35**[1]			**1.32**[1]
Arsenic	**0.014**[3]					**0.029**[3]			**0.037**[3]
Boron	0.08					0.15			0.17
Cadmium	<.0002					<0.0002			<0.0002
Chromium	<.002					<.002			<.002
Copper	**0.0212**[1]					0.0025			**0.0182**[1]
Iron	**3.43**[1]					**0.55**[1]			**2.53**[1]
Lead	**0.0099**[1]					0.0003			0.0026

Manganese	0.163					0.031			0.079
Mercury, μg/L	0.05					<0.05			<0.05
Nickel	0.0329					<0.0005			<0.0005
Selenium	0.0002					<0.0002			0.0002
Vanadium	0.011					0.017			0.012
Zinc	0.023					0.019			0.019
PAKOWKI LAKE, WEST OF BERM									
	May 28	**June 12**	**June 26**	**July 10**	**July 23**	**August 7**	**August 20**	**September 19**	**October 10**
TP	**0.445**[3]	**0.373**[3]	**0.650**[3]	**0.909**[3]	**1.090**[3]	**0.880**[3]	**0.882**[3]	**0.521**[3]	**0.146**[3]
TDP	0.283	0.279	0.464	0.575	0.908	0.562	0.245	0.195	0.106
TKN	2.04	1.37	2.2	2.04	2.48	2.25	6.75	2.3	1.57
Ammonia	0.18	0.16	0.18	0.11	0.16	0.05	0.06	0.04	<0.01
Nitrite/Nitrate	0.016	0.039	0.127	0.026	<0.003	0.113	<0.003	<0.003	<0.003
TN	2.06	1.41	2.33	2.07	2.48	2.36	6.75	2.3	1.57
DOC	15.1	18.3	21.3	21.7	25.8	30.6	32.8	35.3	22.8
Chloro *a*	16.2	8.0	9.4	31.7	38.7	162.6	427.1	119.4	23.9
Secchi Depth, m	0.1	0.3	0.2	0.1	0.2	0.1	0.2	0.1	0.3
Sulphide							<0.005	<0.005	<0.005
NFR, fixed									7
ETZIKOM COULEE AT SR 855									
	May 28	**June 12**	**June 27**	**July 10**	**September 19**	**October 10**			
TP	**0.326**[3]	**0.959**[3]	**0.137**[3]	**0.168**[3]	**0.427**[3]	**0.195**[3]			
TDP	0.301	0.896	0.116	0.162	0.397	0.137			
TKN	1.38	1.68	1.93	2.5	1.5	1.35			
Ammonia	0.01	0.09	0.18	0.13	0.07	<0.01			
Nitrite/Nitrate	<0.003	0.003	<0.003	0.003	0.01	<0.003			
TN	1.38	1.68	1.93	2.5	1.51	1.35			
Silica	3.6	3.5	9.4	10.9	9.9	2.4			
DOC	14.9	22.3	25	29.4	31.4	19.3			
	830	1640	1640	1750	3126	2398			
NFR	1	4.7	8	4	16	3			
NFR, fixed						<0.4			
MANYBERRIES CREEK SOUTH OF ORION									
	May 28	**June 12**	**June 27**	**July 10**					
TP	**0.161**[3]	**0.130**[3]	**0.110**[3]	**0.377**[3]					
TDP	0.132	0.128	0.078	0.242					
TKN	1.12	1.18	1.2	1.11					
Ammonia	<0.01	0.04	0.04	0.15					
Nitrite/Nitrate	<0.003	<0.003	<0.003	0.007					
TN	1.12	1.18	1.2	1.12					
Silica	9.1	6.35	3	23.2					
DOC	16.3	20	21.3	17.1					
NFR	1	2.7	2	7					

Water Quality Guidelines:
[1] CWQG for the Protection of Freshwater Aquatic Life
[2] CWQG for Agricultural Uses
[3] AWQG

Appendix II. Seasonal succession of phytoplankton in Pakowki Lake (central and west of berm basins), May-October, 1996. (Prepared by Bio-Limno Research and Consulting).

At the beginning of the season (May), the phytoplankton assemblage in both basins consisted primarily of *Phormidium* spp. (a non N-fixing cyanobacteria: Fig. 1), green algae (Fig. 2) and the diatom *Cymbella sileciaca* (Fig. 3). The biomasses of *Phormidium* and *C. sileciaca* during this period were among the highest recorded during the sampling season, whereas the biomasses of green algae were among the lowest.

The summer phytoplankton in both basin was dominated almost exclusively by the N-fixing *Aphanizomenonflos-aquae*, constituting >90% of the total phytoplankton biomass in August. *Ap. flos-aquae* was also the most abundant phytoplankton in Pakowki Lake; biomass was, however, 13 times higher in Central Basin than in West of Berm Basin (Fig. 1). *Anabaena* spp., another N-fixing cyanobacteria, occurred in extremely low amounts compared to *Ap. flosaquae,* but had higher biomass in West of Berm Basin.

Ap. flos-aquae disappeared from the phytoplankton by September and was replaced by the diatoms, *Cyclotella meneghiniana* and *Surirella brightwellii*. *C. meneghiniana* was found in both basins, but in larger amounts in West of Berm (biomass of *C. meneghiniana* in September, when the highest population was recorded, was over three orders of magnitude higher in West of Berm than in Central Basin). *S. brightwelii* was found in West of Berm, but was absent in Central Basin (Fig. 3). The other less dominant diatoms (see Table of Results) tended to be more abundant in West of Berm Basin.

Among the green algae, *Crucegenia quadrata and Scenedesmus* spp. occurred fairly consistently in the two basins over the field season (Fig. 2). In both basins, *C. quadrata* increased gradually with time to a peak in July-August, and then declined, although biomass tended to be higher in West of Berm Basin. The highest biomass of *Scenedesmus* in both basins was recorded towards the end of the sampling season, but in higher amounts in West of Berm than in Central Basin. *Pediastrum,* which occurred sporadically in both basins, had higher biomass that the other green algae by virtue of its large biovolume (this taxon constituted < 4% of total cell numbers). Euglenophyceae (made up of several species: see Table of Results) occurred in substantial amounts towards the end of the season; biomass of all species combined was higher in West of Berm Basin (10395 and 11926 mg m^{-3} in September and October, respectively) than in Central Basin (0 and 17480 mg m^{-3} in September and October, respectively). The Euglenophyceae constituted between 1 and 5% of total cell numbers.

In conclusion, Pakowki Lake was *Aphanizomenon*-dominated during the summer, with biomass being higher in Central Basin. Overall, green algae constituted a small portion of total phytoplankton biomass, compared to cyanobacteria and diatoms. The typical spring diatom bloom was absent from Pakowki Lake. Instead, the diatom bloom *(C. meneghiniana* and S. *brightwellii)* was observed in September-October, with populations being remarkably higher in West of Berm than in Central Basin.

Fig. 1. Seasonal trends of dominant cyanobacteria in Pakowki Lake during 1996 (May-October)

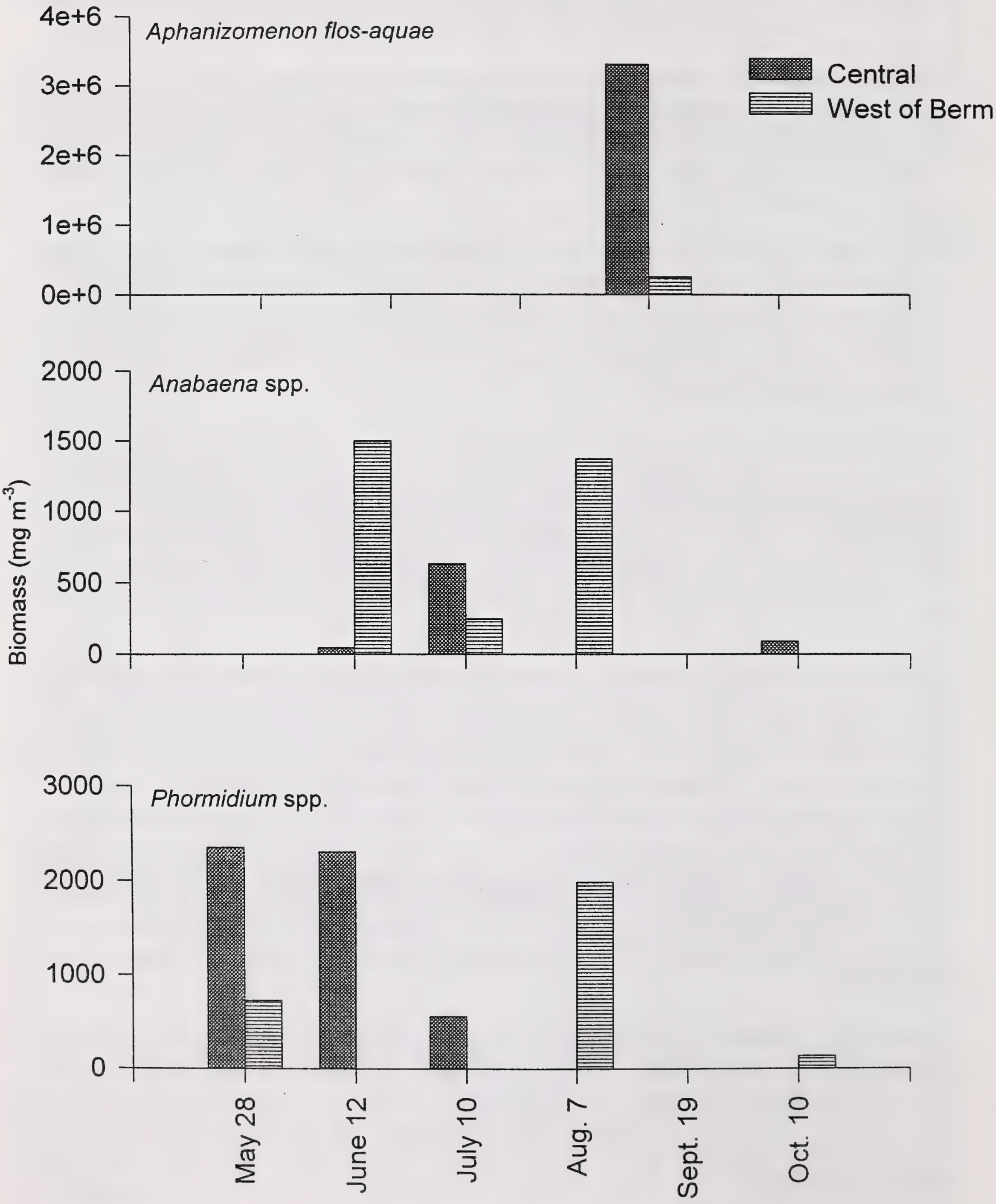


Fig. 2. Seasonal trends of dominant green algae in Pakowki during 1996 (May-October)

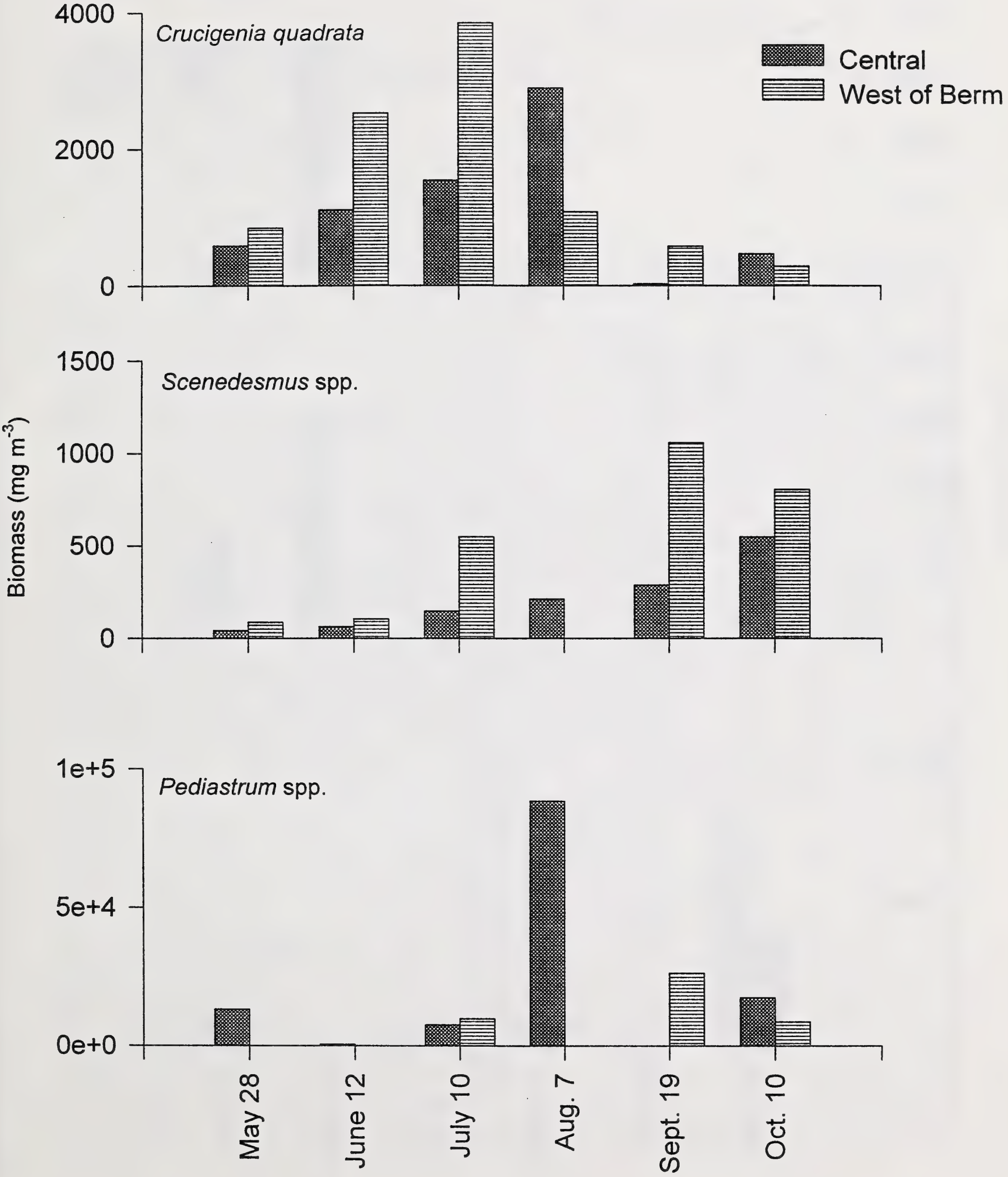
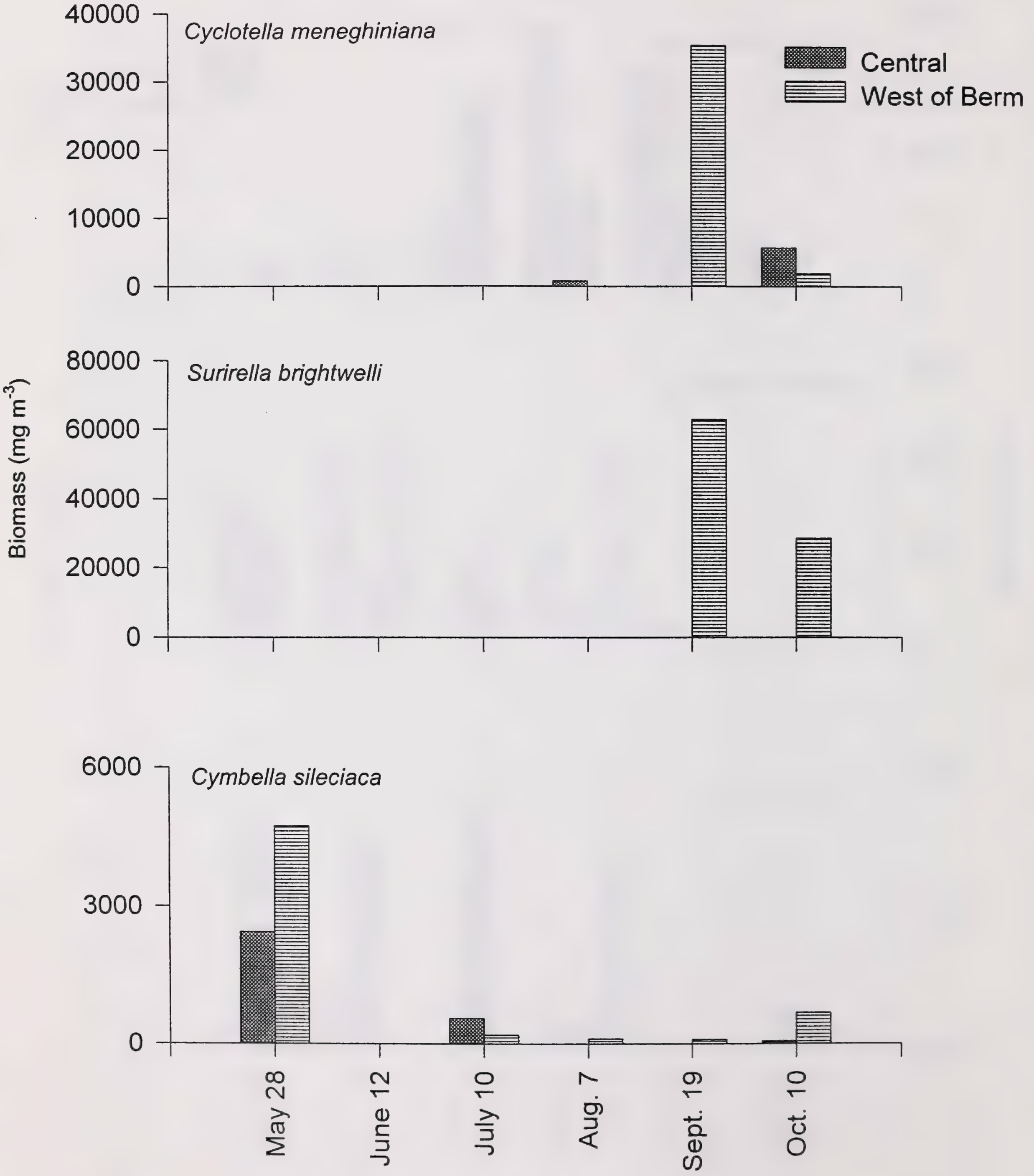


Fig. 3. Seasonal trends of dominant diatoms in Pakowki Lake during 1996 (May-October)



Appendix II. Continued.

QAQC ANALYSIS BY C. EARLE
PAKOWKI LAKE, WEST OF BERM, AUGUST 7, 1996
DATE ANALYSED: 12-15-1996
SUBSAMPLE VOLUME: 0.2 mL
TRANSECT WIDTH: 970 m
COUNTING AREA: ROUND
CHAMBER LENGTH: 26 mm

TAXON	CELLS (L^{-1})	BIOMASS ($mg.m^{-3}$)	COL	CELLS	LEN	WID
Aphanizomenon flos-aquae Ralfs	24420320	67991.1689	232	5393	6.1	5.0
Oscillatoria limnetica Lemm.	374256	143.8469	3	36	5.8	2.5
Merismopedia minima Beck	421038	17.2696	4	612	0.8	0.0
Snowella littoralis (Hey.) Komarek & Hindak	105260	94.7268	1	110	2.5	0.0
Chroococcus dispersus (Keiss.) Lemm.	947336	257.2459	9	63	4.2	0.0
Anabaena circinalis Rabenhorst	105260	165.3724	1	12	6.3	0.0
Ankistrodesmus setigerus (Schroeder) West	526298	50.2224	0	5	40.5	3.0
Oocystis Borgei Snow	105260	213.8146	1	7	10.4	7.3
Crucigenia quadrata Morren	315779	36.1535	3	20	3.9	2.9
Monoraphidium contortum (Thuret in Breb.)	105260	7.2651	0	1	39.0	2.6
Oocystis lacustris Chodat	210519	202.089	2	10	10.9	5.8
Elakatothrix gelatinosa Willen	210519	11.9257	2	3	18.4	2.8
Tetraedron minimum (A.Braun) Hansgirg	105260	22.937	0	1	7.6	7.4
Monoraphidium minutum (Naegeli) Komarkova	947336	59.0393	9	10	13.5	2.3
Scenedesmus quadricauda (Turp.) Breb.	736817	115.2363	7	20	12.1	2.4
Oocystis submarina v. variabilis Skuja	105260	30.3567	1	4	6.8	4.5
Scenedesmus acuminatus (Lager.) Chodat	105260	11.2344	1	4	13.0	2.8
Euglena elastica Prescott	105260	223.4727	0	1	46.8	7.6
Phacus caudata Hubner	421038	1239.819	0	4	35.5	17.8
Cryptomonas erosa Erh.	105260	48.7056	0	1	15.3	7.6
Pennate diatom species #1	105260	38.2747	0	1	17.8	5.1
Pennate diatom species #2	210519	12.3146	0	2	9.5	2.8
Centric diatom (13mm diameter)	210519	173.341	0	2	6.3	12.9
Synedra (species unknown)	210519	29.123	0	2	67.4	2.8
Centric diatom (8mm diameter)	526298	78.7884	0	5	3.3	7.6
Pennate diatom species #3	315779	18.2289	0	3	24.5	3.0
Pennate diatom species #4	105260	3.3055	0	1	15.3	2.8
Nitzschia (species unknown)	105260	351.1849	1	1	118.0	6.0

Appendix II. Continued.

Phytoplankton list and biomass (mg/m^3) in Pakowki Lake (Central Basin and West of Berm Basin) in 1996.

TAXON	MAY 28		JUNE 12		JULY 10		AUGUST 7		SEPTEMBER 19		OCTOBER 10	
	C	W	C	W	C	W	C	W	C	W	C	W
Cyanobacteria												
Anabeana circinalis				1498	630	247						
An. flos-aquae								1370				
An. aequalis			45									
An. solitaria											91	
Aphanizomenon flos-aquae					914		3304953	255211	7233	4670	8426	
Aphanocapsa sp.				135		406			132			
Coelomoron microcystoides	19		421		152	1485			152	77	76	
Chroococcus limneticus							4331		3970	2888	6497	
C. turgidus									493			
Gomphosphaeria aponina				23					17			
Limnothrix sp.										19		
Merismopedia tenuissima								1322		211		70
Microcystis incerta									131			
Phormidium amoena												17
P. formosum	1923	476	2221		347			1982				
P. willei	423	253	76		211							118
Pseudanabeana galeata												119
Pseudanabaena spp.										172		
Synechococcus nidulans							1083		235			
Chlorophyceae												
Actinastrum hantzschii										99	28	52
Chamydomonas sp.			183									36
Coelastrum microporum	86			90	45				45			
Cruceginia quadrata	586	848	1112	2527	1543	3844	2888	1083	27	578	469	298
Gloecystis planctonica											264	
Kerotoccocus suecicus			11	44	11	88			11	132	44	
Monoraphidium acuatum								551			34	
Monoraphidium circinale						26				119	157	396
M. grifithii									50	79		67
M. komarkovae										44		
M. indicum										16		53
M. minutum											3	
Nephrocytium lunatum									53			
Oocystis elliptica	108	217				123						
O. rhomboidea				34						35	106	
O. submarina	141			106	352				238			
O. solitaria					17							
Pediastrum boryanum			551		7737	8812					17624	
P. duplex	13218					1102	88121		122	26436		8812
Scen us abu												

TAXON	MAY 28		JUNE 12		JULY 10		AUGUST 7		SEPTEMBER 19		OCTOBER 10	
	C	W	C	W	C	W	C	W	C	W	C	W
Scenedesmus acuminatus											127	42
S. bijuga	21	42	21		42		211				105	
S. bernardii	21											
S. bicaudatus			42		42							21
S. dimorphus										107		
S. incrassatulus				42	21	487			42	84		
S. opoliensis											85	254
S. quadricauda		43		63	42	63			244	867	232	402
Euglenophyceae												
Euglena sp.										5330		416
E. intermedia												596
E. proxima											17480	9041
E. sanguinea												2143
Lepocinclis acuta										2980		
Lepocinclis sp.	297									298		
Phacus acuminatus	127									1787		
Cryptophyceae												
Cryptomonas borealis										698		
C. erosa										298	298	1498
C. reflexa	149	596	447									2143
Cryptomonas spp.												92
Rhodomonas minuta												
Bacillariophyceae												
Achnanthes minutissima		5									65	48
Amphora sp.												202
Cocconeis placentula											4972	16747
Craticula halophila	273	692	273		242			3938		1408	61	1212
Cyclotella meneghiniana					20		793		10	35439	5659	1929
Cymbella sileciaca	2435	4728	68		564	196		109		98	61	682
Diatoma vulgare												1515
Fragilaria ulna			303									
Fragilaria sp.					43					30		
Gomphonema augur		121	20		81			379				101
Gomphonema spp.												259
Navicula cryptotenella											980	21320
Navicula spp.											43	
Nitzschia acicularis												565
Nitzschia amphibia			49									
N. sigmoidea		48								95		
Surirella brightwellii										62944		28611
S. constricta												954
TOTAL	19827	8069	5843	4562	13056	16879	3402380	265945	13205	148038	63987	99962

Appendix III. Analysis for anatoxin-a in algal material collected in Pakowki Lake, 1996. (Prepared by S. Kenefick, Okanagan University College).

Anatoxin-a Analytical Method:

The extraction method developed by Himberg (1989) was modified slightly for the analysis of anatoxin-a. Approximately 50 mg of freeze-dried material was homogenized and mixed with 25 mL methanol and 0.25 mL concentrated hydrochloric acid. The algal cells in suspension were analysed in an ultrasonic bath for 15 minutes and then were centrifuged. The extraction was repeated on the centrifuged pellet and the combined supernatants were filtered though a Gelman 0.2 μm Acro syringe filter. The solution was then evaporated nearly to dryness under a gentle flow of prepurified nitrogen.

The extraction residue was then dissolved in 1 mL chloroform and transferred to a 10 mL culture tube. The solution was then subjected to acetylation derivatization by adding 1 mL glacial acetic acid and 0.5 mL acetic anhydride. The mixture was mixed gently, capped tightly and heated at 50°C for 16 hours. The derivatized solution was evaporated until all acetic acid was removed and was then dissolved in 1 mL chloroform and applied to a prepared silica solid phase extraction cartridge. The cartridge was washed with 7.5 mL acetone-chloroform (5-95) and then acetylation products were eluted with 2 mL methanol. The methanol solution was evaporated nearly to dryness and resuspended in exactly 1 mL chloroform.

This chloroform solution was then used for GC/MS analysis for the acetylanatoxin-a. Immediately prior to analysis, 5 μL of 1-acetylpiperidinewas added as an internal standard. The GC/MS analysis was carried out using selected ion monitoring,. The column used was a DB-1 (30 m x 0.25 mm I.D. x 0.25 μm film thickness). Detector temperature was 250°C, injector temperature was 240°C and the oven was temperature programmed for 80 to 275°C at 15°C per minute. A 1 μL injection was used with a column pressure of 70 kPa. Ions of $m/z = 207,165,164$, and 136 were monitored for acetylanatoxin-a and ions $m/z = 84$ and 127 were monitored for the internal standard. Ions m/z - 165 and 84 were used for quantilation. Glassware blanks were used to check for contamination and standard anatoxin-a solutions were analyzed to confirm accuracy of the method.

Reference:

Himberg, K. (1989). Determination of anatoxin-a, the neurotoxin of *Anabaena flos-aquae* cyanobacterium, in algae and water by gas chromatography-mass spectrometry. Journal of Chromatography, 481: 358:362.

Appendix III. Continued.

Anatoxin-a Analysis Results

SAMPLE		MASS ANATOXIN-a per g of dry biomass (μg/g)
May ?, 1996	Central Zone	N/D*
May ?, 1996	West of Berm	N/D
June 12, 1996	Central Zone	N/D
June 12, 1996	West of Berm	N/D
June 25, 1996	Central Zone	N/D
June 25, 1996	West of Berm	N/D
July 10, 1996	Central Zone	N/D
July 10, 1996	West of Berm	N/D
July 23, 1996	Central Zone	N/D
July 23, 1996	West of Berm	N/D
August 7, 1996	Central Zone	N/D
August 7, 1996	West of Berm	N/D
August 20, 1996	Central Zone	N/D
August 20, 1996	West of Berm	N/D
September 19, 1996	Central Zone	N/D
September 19, 1996	West of Berm	N/D
October 10, 1996	Central Zone	N/D
October 10, 1996	West of Berm	N/D
Manitoba Algae #1		N/D
Manitoba Algae #2		N/D

* N/D = Not detected with detection limit of 40μg anatoxin-a / g dry biomass

Appendix IV. Pesticide and herbicide scan by AEC on sample collected Oct. 10, 1996.

ALBERTA ENVIRONMENTAL CENTRE ORGANICS ANALYSIS DATA SHEET AEC SAMPLE NUMBER: T96-2084

Client : WALKER
SmpNo : 96SWC40604 GrpSmpNo : 96SWC40600
StaNo : AL05AF0060 ProjNo : ABS026
Comment: PAKOWKI LAKE CENTRAL ZONE, COMPOSITE

SmpDate: 10-Oct-96 @ 0930 Samplers..ID1 : 65134
EndDate: @ ..ID2 :

PESTICIDE ANALYSIS (LTR)
METHOD: A114.0
SCAN: LTR

	TimeLines (days) from sample date Max	Actual
Date Received : 16-Oct-96 by: LU	-	6 --
Date Extracted: 30-Oct-96 by: DH	10	20 *
Date Analyzed : 8-Nov-96 by: DH	20	29 *

Raw DataFile : 12084.

VMV_CODE	COMPOUND NAME	ug/L	flag	MDL	VMV_CODE	COMPOUND NAME	ug/L	flag	MDL
100667	2,4-D	.096		.005	100668	2,4-DB	0.000		.005
100669	2,4-DP	0.000		.005	100674	Atrazine	0.000		.005
100675	Bromacil	0.000		.030	100676	Bromoxynil	0.000		.005
100677	Carbathiin (Carboxin)	0.000		.100	100684	Chlorpyrifos (Dursban)	0.000		.005
100688	Clopyralid (Lontrel)	0.000		.020	100678	Cyanazine	0.000		.050
100679	Diazinon	0.000		.005	100680	Dicamba (Banvel)	0.000		.020
100681	Diclofop-methyl (Hoe Grass)	0.000		.020	100682	Disulfoton (Di-Syston)	0.000		.200
100683	Diuron	0.000		.200	100685	Ethalfluralin (Edge)	0.000		.005
100686	Ethion	0.000		.100	100687	Guthion	0.000		.200
100690	MCPA	0.000		.005	100691	MCPB	0.000		.020
100692	MCPP (Mecoprop)	0.000		.005	100689	Malathion	0.000		.050
100694	Phorate (Thimet)	0.000		.005	100693	Picloram (Tordon)	0.000		.005
100695	Terbufos	0.000		.030	100696	Triallate (Avadex BW)	0.000		.005
100697	Trifluralin (Treflan)	0.000		.005	100670	alpha-BHC	0.000		.005
100671	alpha-Endosulfan	0.000		.005	100672	gamma-BHC (Lindane)	0.000		.005
100673	p,p'-Methoxychlor	0.000		.030					

Zero (0) values indicate that the analyte is not DETECTED. MDL - Method Detection Limit

flags B - This analyte is found in the blank as well as the sample. The blank value has been subtracted.
X - Estimated value. The target compound meets the identification criteria, but is less than the MDL.
Q - Qualifying ions present but failed the ion ratio limits.
M - This value is calculated by an alternate Raw DataFile.

* - asterik following the value for Actual days taken indicates the prescribed time for that event was exceeded.

** - the Date Sampled is unknown, therefore timeline calculations can not be performed.

Certified For: R.D. Smillie

Date: 13-Nov-96
Contact Person: Grant Prill

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If there are any questions or concerns regarding this report, please contact the person indicated above.
Please check the mailing information and inform the lab if changes are required.

ALBERTA ENVIRONMENTAL CENTRE   ORGANICS ANALYSIS DATA SHEET   AEC SAMPLE NUMBER: T96-2084

PESTICIDE ANALYSIS (LTR)

Client : WALKER		METHOD: A114.0	TimeLines (days)
SmpNo : 96SWC40604	GrpSmpNo : 96SWC40600	SCAN: LTR	from sample date
StaNo : AL05AF0060	ProjNo : ABS026		Max Actual
Comment: PAKOWKI LAKE CENTRAL ZONE, COMPOSITE		Date Received : 16-Oct-96 by: LU	- 6 --
		Date Extracted: 30-Oct-96 by: DH	10 20 *
SmpDate: 10-Oct-96 @ 0930	Samplers..ID1 : 65134	Date Analyzed : 8-Nov-96 by: DH	20 29 *
EndDate: @	..ID2 :	Raw DataFile : 12084.	

TENTATIVELY IDENTIFIED COMPOUNDS // COMMENTS	ESTIMATED CONCENTRATION
NO ADDITIONAL COMPOUNDS DETECTED	

Laboratory's comments regarding this sample:

The following items regarding the sample were recorded. A Yes notation indicates a problem with the specified item.

Inappropriate Sample Container - No
Inappropriate Temperature - No
Inappropriate Headspace - No
Broken / Leaking Container - No

This sample was analyzed by GC/Ion Trap MS using full scan.

Estimated concentrations for tentively identified compounds are calculated assuming an equal response to internal standards.

* - asterik following the value for Actual days taken indicates the prescribed time for that event was exceeded.

** - the Date Sampled is unknown, therefore timeline calculations can not be performed.

Certified For: R.D. Smillie

DH

Date: 13-Nov-96

Contact Person: Grant Prill

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